JOHNS HOPKINS UNIVERSITY,

BALTIMORE.

STUDIES

FROM THE

BIOLOGICAL LABORATORY

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VOLUME III.

PUBLISHED BY N. MURRAY,

JOHNS HOPKINS UNIVERSITY.

MARCH, 1884—FEBRUARY, 1887.

PRESS OF ISAAC FRIEDENWALD,
BALTIMORE, MD.

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CONTENTS.

THE SIGNIFICANCE OF THE LARVAL SKIN OF DECAPODS. By H. W. CONN, Assistant in Biology.

If there is any one group of animals particularly favorable for the study of Phylogeny and its principles, that group is the Crustacea. Owing to the complete metamorphosis, and the great variety of the lines of development which Crustacean larvae present, they give us the best possible chance for making cross comparisons and correcting errors. From the free swimming condition of the larvae we may conclude that they are in much the same circumstances under which their ancestors were placed; and one serious source of error is therefore avoided, viz. the modification of the embryo by protection, and by being supplied with food during embryonic growth. To be sure, a second source of error has been introduced. The larvae are of course subject to the circumstances affecting them as free swimming forms, and are therefore in many cases modified by their environment; but this error is less difficult to deal with than the former one. Further, the comparative ease of obtaining and studying Crustacean embryos makes them a particular object of interest to a naturalist at the seaside, and consequently subject to strict and exhaustive investigation. Again, the possession of a hard shell makes Crustacea well adapted for preservation as fossils, and we find that representatives of this group are abundant in palæontological collections. All of these facts together make it evident that no group of animals offers such a hope for the solution of biological problems connected with the origin of species as the quite circumscribed group of Crustacea. A consideration of this fact leads me to publish the following observations and suggestions, in hope that they may be of some value as evidence in assisting our understanding of the morphology of the Malacostraca. I am aware that these suggestions are mostly speculative, but such speculations are not without their value, provided that they are considered only as speculations. It is largely by the testing of the truth or falsehood of hypotheses that the truths of nature are discovered.

The study of the egg embryology of the higher Crustacea, and a comparison of the same with the metamorphosis of those forms which hatch from the egg in a very primitive stage, soon convince us that egg embryology can give us little information concerning the early metamorphosis of this group. It is generally admitted that Crustacea have been derived from a nauplius form. A close observation of the egg embryology does, even in higher Crustacea, reveal traces of this stage; but it may safely be stated that, were it not for the fact that certain of the Macroura (Penaeus Lucifer, Euphausia) hatch from the egg as nauplii, no very great importance would have been placed upon the presence of the egg nauplius. Seeing, however, from the study of Lucifer, etc., that some of the higher developed Crustacea begin their line of metamorphosis with the nauplius, the presence of three appendages in the early egg embryo gains significance. It is evident, however, that more information can be gained as to the history of the Crustacea from the study of the larval metamorphoses, than from the egg embryology, however carefully observed. The metamorphosis of Penaeus or Lucifer teaches more than the egg embryology of all the rest of the group. It is of course then very desirable to obtain a knowledge of the metamorphoses of the higher members in as early stages as possible.

Now, as a rule, the higher Decapods hatch from the egg in a form known as zoea, and it therefore seems impossible to trace their metamorphosis back any further, except by the study of the egg embryology. But there is a method of doing this which, while open to certain objections, will in many cases lead to important results. It has been known since the appearance of a paper by Du Cane in 1839, that most Crustacea when they hatch from the egg are surrounded by a very thin, delicate membrane, known as the larval skin. Although this larval skin has been observed in a great many instances, it is not until the last decade that its presence has been considered as of any morphological significance. The study of this cuticle has of late, however, led to some interesting results, and it seems probable that further conclusions of value can be drawn from more extended studies. For it is evident, upon a little consideration, that this larval cuticle is to be considered as a remnant of the cast-off skin of the stage immediately preceding the zoea. If the crab hatches as a zoea, its larval cuticle will give us means of studying the stage

immediately preceding the zoea. A somewhat unsatisfactory method it is, since we cannot expect the larval cuticle to preserve very exactly the form of the earlier larva, but still a method which bids fair to give some results.

The notices of this larval skin, as far as I have been able to find them, are as follows: The first mention of the structure was by Du Cane,[1] who figures it in the larvae of certain prawns and shrimps. Spence Bate[2] in his valuable monograph on the development of Carcinus maenas, describes the larval skin. He says that it is closely applied to and entirely conformable with the body of the zoea, which hatches from the egg. In this he was in error, for later observations on the same species[3] have shown that there is no such conformity. It is somewhat strange, therefore, that he should even in his latest papers still persist in his old mistake.[4] Fritz Müller[5] mentions the larval skin of Acheas and Maja, showing that it is not conformable to the zoea. He first suggested that it was of some morphological importance, calling attention to the fact that the skin found in these crabs shows a considerable resemblance to the tails of shrimps and prawns. He did not attempt to carry the suggestion further. Gerbe[6] simply mentions the presence of a larval cuticle in marine forms in general. Dohrn[7] in his paper on the development of Arthropods, gives an excellent figure of the larval tail of the crab Portunus, with the zoeal tail inclosed. He also mentions and figures the larval covering of the antennae, showing that, as he says, the first antenna is covered by a very large larval skin, which is prolonged into a number of long lobes. He confounds the two pair of antennae, however, and really describes the second pair. He does not attempt to draw any conclusion, or to place any particular significance upon the embryonic skin. Stuxburg[8] mentions the cuticle, but his paper I have not seen. Claus[9] in

[1] Du Cane. Annals and Magazine of Natural History, 1839.

[2] Spence Bate. Phil. Trans. of Royal Society, 1859.

[3] Faxon. Bulletin of Museum of Comp. Anat., Vol. VI, 1880.

[4] Spence Bate. Report of British Association, 1875–1878.

[5] Fritz Müller. Facts for Darwin. London, p. 59.

[6] Comptes Rendus, LIX, 1864, p. 1102.

[7] Dohrn. Zeit. f. Wiss. Zool. XX, 1870.

[8] Stuxburg. Of vas. Kongl. Vetensk. Akad. Förhandl. XXX, 1873.

[9] Claus. Untersuchung zur Erforschung die genealogischen Grundlage des Crustacean-systems, pp. 62, 1876.

his monograph figures the larval tail of Maja, showing that it has seven spines on each side, and that the third spine from the centre is the longest, while the fourth spine is the shortest. He also shows that it is this short fourth spine which corresponds to the two processes of the fork in the zoea tail which is inclosed within the cuticle. He agrees with Müller in finding here a resemblance to the larva of Penaeus, but says nothing more about it. Joli[1] figures the zoea of Caridina just before it hatches, as surrounded by its cuticle, which is closely adherent to the zoea, and presents no peculiarities. Faxon[2] gives a similar figure of Palaemonetes. In another paper[3] Faxon describes more minutely than had previously been done, the embryonic skin of Carcinus and of Panopeus. His figure of the embryonic tail is similar to that of Claus and others, a forked tail with fourteen spines. He points out the errors of Spence Bate, as well as that of Dohrn, in confounding the two antennae. This peculiar form of antenna he considers as the remnant of a stage when they were used as locomotor organs, "as in the Nauplius," finding here a second reminder of an earlier stage. Packard[4] speaks of the larval skin of Gelasmus as presenting no peculiarities, but admits that he is not certain upon the point. Porcellana is figured by Brooks and Wilson,[5] and its larval skin is said to be exactly conformable to the enclosed zoea. Berge[6] gives a figure of the cast-off larval cuticle of the zoea Panopeus, leaving out of his description the antennae, but in other respects his figure agrees with that of Faxon.

A paper by Paul Meyer[7] upon our knowledge of the zoea form requires more attention. Meyer made an extended comparison among the tails of different zoeas, and also as far as possible of

[1] Joli. Development and Metamorphoses of Caridina Demarestii, Ann. Sci. Nat. XIX.

[2] Faxon. Development of Palaemonetes vulgaris. Bull. Mus. Comp. Zool., Sept. 1879.

[3] Faxon. On Some Points in the Structure of the Embryonic Zoea. Bull. Mus. Comp. Zool., Sept. 1880.

[4] American Naturalist, Oct. 1881.

[5] Brooks and Wilson. The First Zoea of Porcellana. In these Studies, Vol. II, 1881.

[6] Berge. Development of Panopæus Sayi. In these Studies, Vol. II, 1883.

[7] Paul Meyer. Zur Entwicklungsgeschichte der Decapoden, Jen. Zeit. XI.

their embryonic cuticle, and reaches a result of considerable importance. From his comparisons he concludes that the original stem to which the Decapods, or at least their zoeas, are to be referred, possessed a forked tail with seven feathered spines on either side. He finds in many Macroura examined by him, that whereas the zoeas may differ widely from this primitive form, the larval cuticle which covers the zoea does exhibit in a remarkable manner a tendency to develop this forked tail with seven spines. Perhaps the most interesting case is that of Dorippe. The zoeal tail of this genus is enormously long, and has two forking processes with two short thorns. But the larval skin shows most beautifully the ordinary forked tail with its seven spines, all but two of which are entirely lost with the loss of the embryonic skin. Meyer shows quite conclusively, if we can base conclusions on evidence of this sort, that this forked tail (Gabelschwantz) is a very widely distributed form, and probably deserves claim as a form from which the other modified tails have descended. Meyer, however, goes further than this; considering the shape of the zoea as a good point for systematic classification, he attempts to remodel the classification of the Crustacea according to the peculiarities of the zoeal tail. From the fact that the Carididae *zoea* possesses seven spines, while other zoeas present modifications of this form, he considers them to have been the earliest branch from the original stem, and believes that they should be placed in a group by themselves, while all the other Decapods should be placed together as an equivalent group. He gives up the name Macroura, or confines it to the Carididae, and divides the rest of the Decapods into a number of groups, for which he has no reason except the number of spines on the zoea tail. That this is hardly justifiable is evident, though it may have some value; but further consideration will show that any classification based on zoeal characteristics must be accepted with great caution.

Meyer shows, however, that very important results may arise from a careful study of the embryonic skin of zoeas. This arises chiefly from the fact that it is impossible to imagine that the peculiar configuration of the embryonic skin can be of any use to the zoea, and that its peculiarities must be due to heredity and not to adaptation. It is for this reason particularly adapted to

the study of the phylogeny of the Crustacea, and also to the study of the significance of the zoea. One interesting suggestion which Meyer draws from his studies is an explanation of the difference between the shape of the crab zoea tail and that of the Macroura. The crab zoea has preserved, he says, the original forked tail, while the Macroura have usually replaced it by a large flattened swimming plate. The Macroura being swimming animals naturally had use for a swimming tail; while the crabs being animals which usually move by walking with their thoracic legs, had no need of a swimming tail, and have not developed anything of the kind. As ingenious as is this suggestion, it is hardly legitimate, at least without considerable modification; for while the adult crab is a walking animal, its zoea is just as truly a swimming animal as is the zoea of the Macroura; and inasmuch as it is the *zoea* which has developed the peculiar telson, the habits of the adult can hardly be considered as the reason for the shape of the zoea. Meyer seems to have overlooked, however, one comparison which the study of larval zoea suggests. This comparison is one of great interest, and it is the object of the present paper to make this comparison evident.

It seems then, as far as can be judged from the published observations, that there are two different relations which the embryonic cuticle may have to the first zoea. It may be closely applied to the future zoea, and conformable to it. It has in this case almost exactly the same shape as the zoea, except that it does not possess spines. At the extremity of the appendages, and at the telsons, the spines of the future zoea are enclosed in a simple sac expansion of the larval skin, as in Fig. 8. This is the arrangement found in ordinary Macrouran development. In these cases it is evident it is impossible to draw any conclusions from the larval skin. Whatever its form was at a time when it represented a free animal, it has become so modified that it is little more than a sac covering the future zoea. If these forms alone had been studied, the larval skin would never have been considered as having any particular significance.

The second form is however more important. In this class we find the embryonic cuticle not conformable to the future zoea, but showing an entirely different shape, often differing very much from the zoea which is enclosed within it. In these cases

it has been found that the caudal plate of the embryonic cuticle shows a distinct fork with fourteen spines, and as far as has been thus far observed this is true whatever be the shape of the zoea tail enclosed within. Differences are also found in the second pair of antennae, which here show a remarkable series of long feathered processes, entirely unrepresented in the antennae of the zoea. In some cases the shape of the maxillipedes may be slightly different (Porcellana). Such modifications are found among the Brachyura; among the Anomoura, and in some Macroura (Callinassa, etc.). A typical instance of the telson thus described is given in Fig. 1, which represents the telson of Panopeus. The embryonic antenna of the same is shown in Fig. 12. Such embryonic zoea as these have evidently retained with some degree of exactness the form of an earlier stage from which the zoea has been derived.

In all the crab embryos which have previously been figured, the embryonic cuticle has been found to present very much the form represented in Panopeus, Fig. 1, and it would be a very interesting fact if this were universal among crabs, since Meyer believes the Brachyura to have branched off from the original Decapod stem earlier than the other groups. But this is not a fact, however. A comparative study of crab embryos shows that the larval skin is not always of the form represented in these figures, but has various other forms. In fact we find a complete series of gradations from the telson of Panopeus, ending in a form which presents but few differences from the enclosed zoea. Since only one of these forms has been figured or described, I will describe four different kinds of larval cuticle observed and studied at Hampton during the last summer.

The first is the ordinary form figured by Dohrn, Faxon, Claus and others. For convenience of comparison I have figured the tail of Panopeus sayi, Fig. 1. The larval telson is prolonged into seven enormously long feathered spines, which are so arranged as to form a forked tail. Of these spines, the fourth and seventh, counting from the centre, are the shortest, and are unfeathered. In Maja (Dohrn[1]) the embryonic spines are nearly of the same length and all feathered. The relation of tail of the future zoea to this larval telson is readily seen from the figure.

[1] Dohrn. *Loc. cit.*

The three inner spines of the cuticle correspond to the three spines within the fork of the tail of the zoea tail, the fourth corresponds to the process of the fork of the zoea tail, and the fifth corresponds to the thorn on the outside of the tail. The sixth and seventh spines are unrepresented in the zoea, disappearing entirely upon the first moult.

The antenna of the same species is given in Fig. 12. It is biramous, with one branch prolonged into four remarkably long feathered spines. The second maxilla I have figured in Fig. 13. I give this figure to correct an error of Faxon,[1] who, in his figure of this appendage, leaves out the exopodite.

The above form of embryonic zoea is the only form which has been figured in crabs. It has been found in Panopeus, Carcinus, Maja, Achaeus and Portunus. Other forms of embryo may be found. In Fig. 2 is represented the embryonic zoeal tail of the edible crab (Callinectes hastata), and in Fig. 14 its second pair of antennae. In this crab the cuticle is thrown off soon after hatching, and the best method of studying it is to extract the embryo from the egg just before hatching. The tail thus found, Fig. 2, resembles that of Panopeus in certain points, but differs from it in others. In both forms we have a forked tail, and in both we have evidence of seven spines. But whereas in Panopeus they are all long and most of them feathered, in Callinectes they are all short and none of them are feathered. The sixth particularly is much reduced, remaining only as a small lobe, which if not carefully looked for will escape notice. As in Panopeus, the relation of the cuticle to the zoea is easily seen, and is the same as in the former species, with the exception of the presence in the zoea of an outside spine corresponding to the seventh embryonic spine.

The antennae of Callinectes, Fig. 3, are interesting from the fact that they have entirely lost the outer very largely developed branch seen in Panopeus. We have here little more than a simple skin to cover the enclosed appendage.

It is evident that in this form the embryonic zoea is removed by a considerable distance from the ancestral type to which the corresponding stage of Panopeus is to be referred. The larval skin

[1] Faxon. Embryonic Zoea. *Loc. cit.*

has lost part of the features inherited by Panopeus, lost part of its independence, and has taken a great step toward becoming a simple larval covering. We might anticipate that one step more would lead to a form in which all traces of ancestral form are lost.

And this step is found to have been taken by the embryos of crabs belonging to the highest group, *e. g.* to the Grapsoida. Figures of the various parts, obtained by extracting the embryo of Sesarma from the egg just before hatching, are given in Plate I, Figs. 5, 6, 7, 8, and Plate II, Fig. 15. Fig. 4 represents the embryonic cuticle of the same some days before maturity. In this genus we see no trace of the highly complex cuticle of Panopeus and Maja, nor any remnant of the stage presented by Callinectes. The seven caudal spines, Fig. 15, have almost entirely disappeared, the only remnant being four small lobes surrounding the two processes of the fork of the zoea tail, and the three inner spines. The processes of the fork of the zoea tail have also lost their likeness to spines, which was so evident in the previously described species (Fig. 1), and are no longer invaginated, but folded at their tips. In all the appendages, Figs. 5, 6, 7, 8, the larval skin is as closely applied to the inclosed appendages as possible, with of course the exception of the spines, which are unrepresented in the cuticle. We find therefore in Sesarma that the larval zoeas have lost almost all traces of any phylogenetic significance and have become little more than delicate sacs enclosing the future zoea.

But we may even go one step farther by studying the embryo of the oyster crab, Pinnotheres ostreum. This crab classed among the Grapsoida, is even in its adult form quite highly modified. But its zoea is still more peculiar, possessing certain features in which it differs from all other crab larvae. The characteristics of this zoea's tail can be seen from Fig. 9, which is drawn at the time of hatching. It has nearly lost its tendency to fork, has lost its outer spines, and has developed a great lobe in the centre bearing a new spine or sometimes two. The whole tail has become much broadened and resembles the tail of Macroura in shape more than is usual. Other modifications of the zoea are still more peculiar. It has completely lost the lateral and dorsal spines so commonly found in crab zoeas, and, as far as I know, it is the only Brachyuran zoea without such spines. The most re-

markable peculiarity is in the complete absence of the second pair of antennae, and in this point it differs from all other Crustacean zoea. The first pair of antennae have become reduced to a minute knob bearing two hairs, while the second pair have disappeared altogether.[1]

The larval cuticle of this interesting zoea, as we might expect, is more highly modified than in any other crab which has been studied. The cuticle of the tail is shown in Fig. 9. It has entirely lost the features characterizing the embryonic zoea of most crabs. The spines have left no trace, and even the forking of the tail has become almost obliterated. In an earlier stage, Fig. 10, the tail is more evidently forked. At all parts of the animal the cuticle is exactly conformable to the future zoea, with the exception of the region of the antennae; and here is the most interesting feature of all. The larval cuticle has *two* well-marked antennae, although the zoea has but one. Fig. 11 shows this point. We see the rudimentary antenna of the future zoea, with its two hairs, and surrounding it is a lobe of the larval skin. Behind this is seen an empty lobe which undoubtedly represents the second pair of antennae. We have here unquestionable proof that in the previous conditions of this zoea two pairs of antennae were present, and that the loss of the second antennae is a larval modification of quite recent date.

We have in this case, since the adult has two pair of antennae, another instance of an appendage being lost and afterwards replaced. Such instances are not rare in Crustacean embryology, and Balfour has used this fact as a link in his argument as to the ancestry of the zoea. As will appear later, however, this modification is undoubtedly a larval modification, and cannot by any method of argument be referred to variation of adults, and it indicates that other cases of disappearance and reappearance are also secondary modifications.

The case of Pinnotheres is also valuable for another reason. It shows conclusively what sort of significance we can place upon

[1] A small figure of this zoea has been given by Semper in "Animal Life as affected by Natural Conditions of Existence" (International Sci. Series). Berge has figured the same larva more minutely in American Naturalist, 1882. He describes a second pair of antennae as rudimentary structures. I have by very careful examination satisfied myself that he is in error upon this point, and that only one pair of antennae are present.

the shape of the larval skin. Every one who accepts evolution in any form will acknowledge that this zoea has descended either immediately or remotely from a form with two pair of antennae, and which possessed other characteristics of the ordinary zoea. Now the fact that we do find these characteristics in the larval skin, though they are not found in the zoea, indicates clearly in what light this larval skin is to be regarded. It is the cast-off skin of the previous stage; modified, it is true, but still preserving more or less the peculiarities of that stage. Indeed we could not expect to find that this cuticle would preserve all of the previous characteristics. It is an embryonic structure, whose only function at present seems to be as a covering for the zoea. It will therefore undergo changes with a tendency to lose all peculiarities which are not of direct advantage to it. But the case of Pinnotheres shows that it may preserve by heredity some of its previous features. The larval skin of Pinnotheres indicates that in a previous stage the zoea possessed two pairs of antennae. In like manner, finding the peculiar tail and antennae present in the larval skin of certain crabs, which gradually becomes simplified as the crab becomes developed, we are justified in concluding that they have some similar significance, and point to a stage which, as a free swimming form, possessed a tail and antennae of a similar character. The question which arises upon reaching this conclusion is, what is this ancestral form of which we see such traces in crab embryos?

Fritz Müller and Claus found an answer to the question in the zoea of Macroura, and undoubtedly they were on the right track. But it will be found convenient and indeed necessary to substitute for their zoea the form known as Protozoea by Claus. Brooks,[1] in his paper on Lucifer, has already hinted at this in his statement, "and we may perhaps see in the larval skin which many crab zoeas shed soon after or even before they leave the egg, and which usually has a conspicuously forked and very spiny telson, a remnant of the unmodified *Protozoea* stage." Taking into consideration all the evidence which can be obtained, there remains little doubt but that this is the true interpretation.

This conclusion is in the first place suggested by the study of the larval antenna. Examination of Fig. 12 will show that

[1] Brooks. A Study in Morphology. Phil. Trans. Vol. 173.

when the larval integument is cast off, a large part of the second antenna disappears. The large branch *b*, such a prominent feature of the larval skin, is unrepresented in the zoea antenna. Evidently the function of the appendage to which the larval cuticle is to be referred was entirely different from that of the zoea antenna. But such a large plumose appendage as that of Fig. 12 must have had a locomotor function, and the larval skin is to be referred to a stage when the antennae were locomotor organs. This conclusion appears in Faxon's[1] paper; but Faxon considers this locomotor stage to be a nauplius. And, indeed, from the study of the antennae, we can get no farther, for the antennae of the nauplius and those of the protozoea are essentially the same, both in form and in function, and whether the larval antennae of crabs is to be referred back to the one or the other is not here evident. In Figs. 21 and 22 are represented the second antennae of a protozoea and of a nauplius respectively; a comparison of Figs. 12, 21 and 22 will immediately show the likeness referred to.

But more important and conclusive evidence can be drawn from the telson of the various protozoeas known when compared with the embryonic zoeas already described. In Plate II. I have figured a number of protozoea tails. Figs. 17 and 18 are the protozoea tails of two species of Penaeus. Fig. 20 represents the same stage of Acetes (?). Fig. 18 is the protozoea telson of Elaphocaris (Sergestes).[2] It will require only a little careful comparison to convince one that the crab larval skin is the protozoea skin. If Fig. 9 be compared with Fig. 15, this with Fig. 2, and then with Fig. 1, it will be seen that we have various steps of a retrograde development, which begins with a form like Fig. 1; and finally if Fig. 1 be compared with Figs. 17–20, the similarity of the embryonic cuticle with the protozoea tail will appear at a glance. In every case we have a forked telson with a number of long plumose spines, typically seven. Occasionally the number may be less. In Fig. 18 there are only six, and this form is much like Callinectes, Fig. 2. In no *protozoea* as far as is known are there more than seven spines; and this is an important fact, since in a large number of *zoeas* the spines increase in number.

[1] Faxon. Embryonic Zoea. *Loc. cit.*

[2] For Figs. 17, 19, 20 and 21, I am indebted to the kindness of Dr. Brooks, who has lent me some of his drawings which were made at Beaufort.

In the later zoea stages of Penaeus there are eight spines; in Gelasimus an extra spine appears in the last zoea stages. In Macroura particularly is this the case, it being very common to find a large number of spines on the telson (Callionassa). Stomatopods also present many instances of the same. Since then no protozoea has more than seven spines, while later stages frequently have a larger number, it is quite evident that the larval cuticle must refer to some form quite like a protozoea. In the typical protozoea there is a forked telson with seven spines on either side, and a large pair of swimming second antennae; and in the larval cuticle of Panopeus there is a forked tail with seven spines on either side, and a large pair of second antennae which were undoubtedly once swimming appendages. Morphological evidence therefore authorizes us in concluding that the two refer to the same phylogenetic stage.

But the probability of such a conclusion appears still greater when we come to consider its significance in enabling us to interpret certain points in Decapod embryology, for it is more than the substitution of the word protozoea for the word zoea. Fritz Müller studying under the impetus given him by the *Origin of Species*, concluded that the zoea[1] was to be considered as an ancestral form, from which all Crustacea which pass through a zoea stage have descended. This conclusion necessitates the belief that in the phylogeny of the group the head and abdomen appeared first, and that the thorax was afterward interpolated. If this be so, our understanding of the history of Crustacea will be greatly affected by it. But there have appeared many reasons why Müller's views must be rejected. Claus has conclusively shown that we cannot accept a zoea as the original stem, but we must believe that the segments of the body have appeared in regular order, from before, backwards; and in such a history a form with no thorax, but well developed abdomen, has no place. But admitting this, it becomes a puzzle what to do with the zoea.

[1] It is hardly necessary to point out that the term zoea, as used by Müller, and as will be seen later as used by Balfour, has a different meaning from the term zoea which Claus considers an ancestral type. Both Müller and Claus saw that the larval cuticle referred to the zoea of Penaeus; but Müller concluded that the typical Decapod zoea with no thorax was the ancestral stem, while Claus found the stem in the zoea of Penaeus, a very different animal. Claus' view is much the same as is argued here with the substitution of protozoea for zoea.

The zoea stage is of almost universal occurrence among Decapods,[1] and is also present in Stomatopods. Upon all principles of embryology then it must have some important meaning.

There is no need of here reviewing the arguments used by Claus for showing that a form like Penaeus zoea was probably the form from which other zoeas descended, beyond pointing out one or two facts. 1. All of the arguments used by Claus apply as well as if the *protozoea* be taken as the first stem form, as they do when the Penaeus *zoea* is the starting-point, for in each case do we have a form with the segments of the thorax developed. 2. While Claus and others have shown by studies on Penaeus, Lucifer, etc., that the protozoea stage must be included in the history of Macroura, studies on crab larvae show equally well that such a stage must also be included in the ontogeny, and therefore probably in the phylogeny of Brachyura. 3. Claus concludes that a form like Penaeus zoea with all its segments, was the original stem of the higher Crustacea, and that the ordidary zoea so commonly occurring in Macroura and Brachyura is a secondary and a *larval* modification of this stem. To this latter statement Balfour objects, and assumes that while the higher Crustacea must have developed their segments in the order which Claus describes, still there probably existed a later ancestral form with a shortened thorax, from which the typical zoeas have descended. Upon this assumption of Balfour's the study of the larval skin of crabs bears some direct evidence.

It may be well just here to mention a few facts which indicate that secondary *larval* modifications of importance do exist in the zoeas, particularly as the points I shall speak of have hitherto not been noticed. I am aware that an embryologist in speaking of larval modifications, and the consequent falsification of the embryological record, is treading on dangerous ground. Eminent biologists have expressed themselves as out of sympathy with this sort of reasoning, considering it as very unscientific. But at the same time there seems no reason to doubt that the term larval modification has a meaning and expresses a fact of nature. Larvae are free swimming animals, and obtain their food by their own exertions. They are, therefore, engaged in a struggle for existence, and are subject to modifications as truly as are

[1] Some crabs are said to be without a zoea stage.

adults, perhaps even more so, since they are infinitely more abundant than are the adults. There is no reason in nature why the environment should not affect larvae as well as adults. The only question is as to our ability, with the evidence at our command, to unravel the confusion without making unwarrantable assumptions. In many cases, however, this can be done in such a manner as to leave no room to doubt as to its correctness. In the instances given in the previous pages we have undoubted proof that such larval modifications do occur in Crustacean development. Take for instance the very evident meaning of the zoea of Pinnotheres ostreum. This zoea has a tail such as is figured in Fig. 9, and, as has already been pointed out, only one pair of antennae. Now all other zoeas, as far as is known, have two pair of antennae; if therefore the zoea does represent an ancestral form, it must be a form with two pair of antennae. We must therefore conclude that the loss of the second antennae is a larval modification, or that Pinnotheres has descended by a special line from a special ancestor, which is of course absurd, and is, if necessary, further negatived by the fact that its larval skin has two pair of antennae. Or again, compare the tail of the same species with the tail of another species of the same genus figured by Fritz Müller.[1] The tail of Müller's species was the ordinary forked tail with three spines, so common to crab zoeas. It very much resembles the tail of Sesarma figured in Fig. 13. It has in fact not the slightest resemblance to the tail of our species. Now even if the zoea did represent an ancestral stage, it must be a stage very far back in the history of the group. But the two species of Pinnotheres are, however, only the very latest diversions, and the great difference in the form of the zoea which such closely related species present cannot possibly be referred back to any difference in a zoeal ancestor. There is no avoiding the conclusion that we have in the peculiarities of Pinnotheres zoea instances of larval modifications.

A more general result of the same kind may be obtained by comparing the zoeas and megalopa of various crabs. Among zoeas there is a very great variety of forms. Compare for instance the zoea of Pinnotheres with that of Panopeus. In one case we see a rounded body with no dorsal or lateral spines, with

[1] Fritz Müller. Für Darwin.

a plate-like tail, with the first pair of antennae rudimentary and the second pair entirely wanting. In the other the body is of different shape, it has enormously long dorsal spines and a long rostrum; its tail is forked, each half carrying four spines, and the antennae, particularly the second pair, are very large and well developed. This is a single instance, but it would be easy to find any number of similar cases, for zoeas of different crabs when compared with each other are exceedingly unlike. But how is it with regard to the next stage? What will be the result of the comparison of a like number of megalopa? I think that every one acquainted with Crustacean embryology will agree that the megalopa are much more alike than the zoeas. Compare for instance the two pair of antennae. While in the zoea stage these appendages present much variation, in the megalops stage there seems to be a uniform type in which all agree. The first pair is short, bent, and has a large ear sac; the second longer, uniramous and jointed, with hairs at the joints. Or again, in the shape of the tail, a point of such variety in the zoeas, the megalops shows wonderful sameness. The shape of the carapace also, while it is somewhat more variable than the other points mentioned, presents a great adherence to a given form. It is long and flattened, usually roughly rectangular, and carrying in front a rostrum of varying length. It is certainly possible to distinguish the megalopa of different crabs from one another, but it is done by the comparison of minor points, *i. e.* size, distribution of pigment, etc. A comparison of a certain number of crabs in their different stages will show that in the zoea stage they are very unlike, often more unlike than in the adults; while at the megalops stage they have again attained a form in which they present few differences. Now this fact, so much at variance with the principle that embryos proceed from the like to the unlike, is only explicable on the supposition that the peculiarities of the zoea are due, not to inheritance from an ancestral type, but to secondary larval modifications.

Some of the peculiarities of the zoea are larval modifications, but how many of them? Are we to consider this fact to be confined to a few, and to the minor points? or are we to extend it to more important ones, and consider the zoea of the ordinary type itself as a larval modification? To the latter question Balfour

has answered no. Admitting the force of Claus' arguments he believed that the Malacostraca are descended from a form with head, thorax and abdomen; but he also thought it probable that a later stage, with no thorax, also existed as an adult form, and from this the various zoea have received their peculiarities by inheritance. He thinks the zoea of different groups too much alike to be explained on the hypothesis of Claus, although their minor differences were thus acquired. Balfour's reasons for believing the zoea to represent an ancestral form are chiefly "the points of agreement in the number and character of the appendages, and the form of the abdomen, etc., between the different zoeas." But what is this agreement? All zoea, with the exception of Pinnotheres, have the antennae, mandibles and maxilla present. But this is not remarkable since the shortening of the zoea body occurred at the thorax and not at the head, nor is the character of the appendages very similar. Compare for instance the antennae of Pinnotheres with those of Panopeus, or with those of Palæmonetes. But as for the rest there is not even uniformity in the number of appendages. Crabs usually have two maxillipedes, but Porcellana has the rudiment of a third. Macroura have three maxillipedes, while in the zoea of Lucifer, Penaeus, etc., the segments and appendages of the thorax are also present, and some of them are also present in other Macrouran zoea. Nor does the likeness in the number of segments appear of any more weight, because in the first place there is no such likeness. The crab zoea has at first five abdominal segments, while the Macrouran zoea has six. But we should, even according to the theory of Claus, expect a considerable uniformity on this point. This theory would probably find that the zoea body of the zoea (protozoea) has been shortened to adapt it better for a swimming life. The most simple method for this shortening would be in a disappearance of the least important part of the body. The head would of course be retained, since it contains important organs. The abdomen would be retained as a swimming organ. This would lead us to expect a relation much as we find it. Again, the likeness in the form of the abdomen is not found. Compare the abdomen of a crab zoea with that of a shrimp and there is indeed almost no likeness. Each consists of a number of cylindrical segments, but that is about the only simi-

larity. The number is not alike, the shape is not alike, the relation to the cephalothorax is not alike, the shape of the telson is not alike. The various likenesses upon which Balfour lays such stress do not then appear very remarkable, and may be all explained by inheritance from the protozoea.

Balfour's reasons for accepting the zoea form as ancestral do not appear very cogent, and there are also important positive arguments against it. He supposes that in the history of the group the whole thorax disappeared, and afterwards reappeared again, and this is evidently difficult to believe. Balfour endeavors to meet the difficulty by supposing that "the larvae of the zoeal ancestors always developed the appendages in question." But this is a simple assumption and does not diminish the difficulty.

Certain other facts make their appearance in connection with the study of the larval skin of the zoea which, when their connection is studied, leave Balfour's theory untenable. It is impossible to consider this larval cuticle in any other light except as the cast-off skin of some previous stage. Now we have seen that this skin is a remnant of some stage resembling a protozoea, and if there had intervened in the ontogeny of the group between this stage and the ordinary crab zoea, a well-marked ancestral stage with no thorax, and with the peculiar structure which Balfour assumes as an ancestral form, it is very remarkable that the protozoea should so universally be represented in the larval cuticle, and never the hypothetical zoeal ancestor. A comparison of Figs. 1, 2, 9 and 13 will show that the cuticle is in all cases either a protozoea form or a degeneration of this form to a simple covering. In none of these cases can it be considered as a zoeal remnant. Now if the zoea did represent an ancestral form, we would much more naturally expect that in the higher modified zoeas we should find the larval skin to give evidence of this ancestral type, rather than to be referred back as far as the protozoea.

Without pressing this point, the same conclusion is reached from another standpoint. Balfour himself would undoubtedly have admitted that, if by a careful study the various differences shown by different zoea could be traced backwards in converging lines till they all met in one form, that this form would

necessarily be considered as the ancestral stem. Now by the facts brought out by the various papers which treat of the larval skin it becomes evident that this can be done. By the results of the work of Dohrn, Claus, Faxon, Meyer, as well as those embodied in this paper, we see that all zoeas in which the previous history can be traced are to be referred back to a form with a forked telson with fourteen spines, and probably a form swimming by its antennae. We can therefore hardly escape the conclusion that from this form, whatever it might have been, the various zoeas have sprung. Now if it be admitted as is argued here that this form is nothing but a protozoea, we have the best embryological proof that the *zoea* are descended from a *protozoea* form, and not from a later zoea ancestor, as Balfour supposes. Or if it be not admitted that this cuticle refers to the protozoea, and be claimed that, as Müller points out, it refers to a zoea like that of Penaeus, the result as far as concerns the meaning of the ordinary zoea will be the same. For Penaeus zoea is not formed after the type of ordinary zoea, but has a complete thorax. In either case then the conclusion is that the zoea of all ordinary Decapods can be traced backwards to a form with its body supplied with a thorax, and from this form the zoeas have diverged. We get therefore no hint of a later ancestral zoeal form with shortened body, which must therefore remain an assumption, against which the facts of embryology speak quite strongly.

Another objection to this conclusion has also appeared. Brooks[1] has pointed out, that if the zoea be a larval modification, we cannot explain the appearance of zoeal peculiarities in Schizopods which are absent in the lower Macroura. If, however, the protozoea be taken as a stem form, this objection hardly seems valid. The presence or absence of a larval peculiarity will not depend upon the degree of development of the adult, except in a very indirect way. It is probable, it is true, that the more highly developed Crustacea will, as a rule, have more highly modified larvae, but at the same time we must remember that it is not the environment of the adult which produces larval modifications, but the environment of the larvae. Now there is no difficulty in believing that the larvae of Schizopods might have

[1] Phil. Trans., Vol. 173.

been placed under circumstances in which a shortened body would be of advantage, and if this were the case there is no reason why the thorax should not disappear here as well as in the higher Crustacea. And, on the other hand, there is no difficulty in seeing that the larvae of certain low Macroura might be under circumstances where a shortened body was not necessary, and the final condition of the adult would not affect these circumstances except very indirectly. If then we start with a protozoea as a stem form, we can see no difficulty in the fact that some lower Crustacea may have a more highly modified larvae than others which are higher in adult classification. The fact then that certain Schizopod larvae possess no thorax, while some of the lower Macroura do possess these segments, is not a difficulty as at first appeared. If further, as Boas[1] has shown to be probable, the Schizopods do not represent a natural group, the difficulty disappears altogether.

Boas in the above-mentioned paper, which is certainly one of the most valuable papers on Crustacean morphology which has appeared for some time, arrives at a conclusion which is somewhat at variance with that suggested above as to the origin of the Decapods. He finds the stem form of the higher Crustacea in the Phyllopods, and derives the Malacostraca directly from them. A careful study of this paper will convince one that Boas has not drawn his conclusions without good reason, and that in the main he is right, at least as to the relation of the various Malacostraca to each other. His classification is certainly a more natural one than has previously appeared, and as far as the relation of the various groups of Malacostraca is concerned there seems great probability that he has reached correct conclusions. But he is hasty in assuming that they are derived directly from the Phyllopods, and indeed gives no good reasons for this assumption. It seems, according to Packard,[2] that there are very strong reasons for disbelieving any such view, and for looking upon the Phyllopods as a quite highly modified form. Boas in his deductions hardly takes into consideration the embryology of the various forms, or at least does not give to this branch of

[1] Boas. Studien über der Verwandschaftsbeziehungen der Malakostraken. Morph. Jahr. 1883.

[2] Packard, A. S. Monograph of North American Phyllopods.

evidence its due weight. He leaves no room in his phylogenetic stem for anything to which the zoea stage can refer.[1] Now if embryological evidence, or rather in this case evidence from metamorphosis, has any significance, there can be no doubt that the long series of protozoeas and zoeas must have some meaning, and Boas is not justified in leaving them out of consideration as he has done. All of the results of Boas are reconcilable with the teachings of embryology, if we make a slight change. He derives by direct descent from Phyllopods, a form of Schizopod (Euphausia), and from this point on the one hand he derives the Decapods, and on the other the remaining Schizopods, Isopods and Amphipods. Now if we substitute for the Phyllopods in this scheme a form equivalent to a protozoea, we have at the same time reconciled the deductions of Boas as to the relations of the Malacostraca, the conclusions of Packard as to the position of Phyllopods, and the evident teachings of embryology of Decapods, Schizopods, etc. The conclusion obtained by the study of embryology as here explained is not at variance with those of Boas obtained by a comparative study of the adults, except as to the relation of Malacostraca to the Phyllopods, and in this respect it is in coincidence with results of Packard's study on the Phyllopods.

It is always interesting to take into consideration what might have been the reasons for the various changes that embryology shows have taken place, even if there is no great probability that our reasons are the actual ones which produced these changes. Such speculations do enable us to get a better insight into the workings of nature and help us to interpret her mysteries. It may not be an idle question, therefore, to ask, how did this larval modification which produced the zoea from the protozoea come about? The zoea differs from the protozoea principally in the absence of the thorax with its appendages. The body was probably shortened to enable the larva to swim better. The value of great swimming powers in a larval form is evident. A comparison of the swimming of the zoea of a crab or a shrimp with that of the protozoea or zoea of Penaeus, shows immediately that the forms without a thorax are much better swimmers. There are

[1] I have, unfortunately, been unable to obtain Boas' paper upon the Decapods. (Vedensk. Selsk. Schrift. naturw. og. math. afd. 6, R. 1.)

probably two reasons for this. 1. The body is more compact in shape and therefore better adapted for swimming, and the abdomen is so joined to the cephalothorax as to enable it to be used as a swimming organ. 2. A second reason is found in the distribution of the force of development. If all the energy of a developing embryo be concentrated in building up two regions of the body, head and abdomen, it is clear that in a given time these regions would reach a more complete state of development than would be the case if part of the developing energy was turned toward the building of the thorax. In such a case we might expect to find, in the zoea without a thorax, a more highly developed abdomen and head, and a body better adapted for swimming. Here then do we find one reason for the disappearance of the thorax in the larvae. We would get by thus concentrating the developing energies an animal better fitted for the struggle for existence to which it as a free living animal is exposed. Now, a person who has compared the habits of the protozoea with those of an ordinary zoea will be convinced that this probably expresses a truth, for the zoea is much better adapted to struggle with its enemies than is the protozoea. It is quicker in its motions, and has no long, cumbersome locomotor antennae. A protozoea can easily be caught with a glass tube, but it is much more difficult to catch a zoea. Now it is probable that the greater vivacity of the zoea is due to the more perfected development of the parts present, and it is by no means improbable that this may be due to the fact that the thorax is undeveloped, thus enabling the developing energies to be directed toward the perfecting of the rest of the body.

It will also be interesting to make a comparison between the form of a zoea and that of the adult, and to discover, if possible, whether there be any connection between them. The Macrouran zoea has a broad swimming plate for a telson, while the crab zoea has a smaller and a forked telson, evidently not particularly adapted to swimming. Meyer[1] thinks that an explanation may be found in the relative habits of the adults; the crab being largely a walking animal has retained its original forked tail in its zoea, while the Macroura being a swimming animal has developed a swimming tail in its larvae. We can hardly see, however, in

[1] Jen. Zeit. XI.

what way the life of the adult can thus influence the tail of the larva, particularly since it appears that the zoea is a secondary *larval* modification. There may be, it is true, a certain amount of connection between the general form of the zoea and that of the adult. The zoea must eventually change into the adult, and evidently the more the zoea approximates to the adult in form the less radical will be the change. All embryologists have found that it is at the period of moulting that the young zoea are most apt to die, and that the greater the difference between the two stages the greater the difficulty of passing through the moult safely. It follows from this that, other things being equal, natural selection would tend to preserve these larval forms which are most like the adult. Comparing the adult shrimp with the crab, bearing in mind this principle, what differences might we expect to find between their respective zoeas? Evidently that the crab zoea would have a tendency to develop a large cephalothorax and a small abdomen, while the shrimp zoea might possess a small thorax and a large abdomen. It is needless to point out that this is exactly what we do find. The crab zoea possesses an enormous cephalothorax and a small abdomen, the shrimp zoea has a small cephalothorax and a large abdomen. Or another point of comparison may be found in the position in which the abdomen is carried, though it is by no means as certain that it has any meaning. The crab zoea carries its abdomen doubled under its body in a manner which certainly reminds us of the position of the same in the adult, while the shrimp zoea carries its long abdomen stretched out behind the cephalothorax, more as it is in the adult shrimp. Perhaps this can be made more evident by considering what a great metamorphosis a shrimp zoea would be obliged to go through to become converted into a megalops; or still more, to imagine what a very great change would be required to transfer a crab zoea into the first stage of the adult shrimp. It seems quite evident then, that the form of the adult may in some such general way influence quite materially the shape of the zoea, and that this probably accounts for the principal differences in the general form between the zoeas of crabs and the zoeas of Macroura. But beyond some such general effects it is impossible to see how the adult can influence the free swimming larvae. In minor points there can be no traceable

relation. If then the zoea be simply a secondary larval modification, we cannot find an explanation of the shape of the two forms of zoea tail in the habits of the adult animals, and an explanation must be sought for different from that given by Meyer.

An explanation can be found without any very great difficulty, an explanation somewhat similar to that given by Meyer, except that it finds the differences in the tails due to differences in the habits of the zoea themselves, and not of the adults. The Macrouran zoea is a much more powerful swimmer than the Brachyuran crab zoea, and this is a peculiarity which especially fits it for its struggle for existence. There appears in the crab zoea a tendency to develop spines upon the cephalothorax and elsewhere, which spines, as Müller has shown, are for protection. This zoea has therefore become fitted for its struggle for existence by acquiring a coat of defensive armor. This being the case, the crab zoea does not require any very great locomotor organs, though of course it is necessary that as a free swimming animal it should have some rapidity of motion. But the crab zoea does not depend upon flight for protection, and has therefore not developed any powerful swimming tail, but has retained more nearly the protective forked, spiny tail of the protozoea. The Macrouran zoea on the other hand seems to be largely dependent upon the quickness of its motion to escape danger. A shrimp zoea is a remarkably vigorous larva, darting away from its enemies with great rapidity. Not having any protective armor to rely on, it has acquired a swimming tail, the original telson becomes broadened out into a large flat plate, serving as a strong paddle, enabling the larva to escape its enemies by rapid flight. Quite suggestive is the case of Pinnotheres zoea. Here the body has lost all its peculiar protective armor in the shape of spines, and quite closely approximates the shrimp zoea in this particular. A glance at its tail, Fig. 9, will show that its tail has at the same time became broadened out into a swimming plate. Another instance of the same kind is Tatuira, which is in most respects like an ordinary crab zoea, but has no spines and has a broad flat swimming tail. It would seem, therefore, that the differences between the two types of zoeal tail are dependent on the different methods of defence which are exhibited by the two groups: the one group

by acquiring a defensive armor retains relatively unaltered the primitive tail; the other having no such armor has been compelled to seek its protection in flight, and has become the possessor of a broad swimming tail. It is thus possible to explain how the protozoea became modified in the larvae of the different groups of Decapods in different directions; partly by a comparison of the resulting larval zoea with the adult which arises from it, and partly from the fact of the different methods which have been acquired in different groups for protection.

Before leaving the subject, another question arises. We have seen that the zoea is a larval modification which has never been represented in the history of the adults of the race. But how long has it been included in the larval metamorphosis? At the time when the Brachyura and other lines branched off from the original Decapod stem, is it probable that their larval history included a zoea stage? Or are we to believe that at that time the larval history was a direct repetition of phylogeny? Some considerations lead us to the former supposition, without, however, giving us sufficient grounds for accepting it. The fact that the zoea in various groups can be traced back by entirely separate lines to an original protozoea indicates that there was no common zoea form even in the larval history of the remote forms; but it would seem at first sight that it must be assumed that the crabs when they branched off from the Decapod stem possessed a zoeal larval stage. The zoea of crab is a perfectly characteristic form, not for one instant to be confounded with the zoea of any other form, all the crabs conforming to one type. The same is true, though perhaps less forcible, in the ordinary Macrouran zoea, and it would be easy to find an explanation of these facts on the assumption that when the crabs and Macroura branched from the Decapod stem they each possessed in their metamorphosis a zoeal stage, and that consequently the zoeas of their descendants have certain inherited points in common. But while this hypothesis seems enticing, as accounting for the various likenesses as well as unlikenesses shown within any one group of zoeas, it is not a necessary assumption, and cannot therefore be made with much confidence. We have seen that many of the points wherein the zoeas of crabs agree with each other, and differ from those of shrimps, are explicable on other grounds. The relation of the

cephalothorax to the abdomen, and the size of the abdomen, may be largely dependent on the form of the adult; the loss of the thorax is brought about to increase the swimming powers; and the shape of the tail may be dependent on the presence or absence of a defensive armor. All of these modifying circumstances would be present in the environment of all the zoeas of a given group, and the resulting form would naturally in most cases be quite similar. While then the above supposition would be very convenient in enabling us to account for certain universal likenesses found among crab zoeas, etc., we are not at present justified in making it. It may be possible that the zoea is ancestral in the sense that the original crab ancestor possessed this stage in its development as a larval modification, though it was not represented by any adult form. But it is also possible that it is not ancestral even in this sense. At present we cannot decide between the two views.

The study of the larval cuticle of Macroura and Brachyura leads to the following results. All Decapods are to be referred back to a form similar to the protozoea, in which the segments of the thorax, and probably of the abdomen, were present, and whose antennae were locomotor organs. In the larval history of most living Decapods the *protozoea* has become modified by the free life of the embryo, and the result has been the appearance in the embryology of a *zoea* stage; a stage differing quite widely in different groups of Decapods, and which must be considered as a secondary larval modification, and not as an ancestral form.

In conclusion, I wish to express my obligation to Dr. Brooks, who has aided me with valuable suggestions, and has kindly placed many of his drawings at my disposal.

EXPLANATION OF PLATES.

PLATE I.

FIGURE 1.—Tail of Panopeus zoea at the time of hatching, enclosed in its cuticle.

FIGURE 2.—Tail of Callinectes zoea in larval cuticle.

FIGURE 3.—Second antennae of Sesarma zoea.

FIGURE 4.—Tail of Sesarma zoea a number of days before maturity.

FIGURE 5.—First antennae of Sesarma zoea at time of hatching.
FIGURE 6.—Second maxilla of Sesarma zoea at time of hatching.
FIGURE 7.—First maxilla of Sesarma zoea at time of hatching.
FIGURE 8.—First pair of maxillipedes of same.
FIGURE 9.—Tail of Pinnotheres ostreum at time of hatching.
FIGURE 10.—Tail of same a number of days before hatching.
FIGURE 11.—First antennae of same, showing the larval cuticle corresponding to the lost second pair of antennae.

PLATE II.

FIGURE 12.—Second antennae of Panopeus zoea, showing larval cuticle.
FIGURE 13.—Second maxilla of same.
FIGURE 14.—Second antenna of Callinectes zoea within larval skin.
FIGURE 15.—Tail of Sesarma at time of hatching.
FIGURE 16.—Mandible of Pinnotheres zoea.
FIGURE 17.—Tail of protozoea of Penaeus.
FIGURE 18.—Tail of Elaphocaris (Sergestes).
FIGURE 19.—Tail of a second species of Penaeus protozoea.
FIGURE 20.—Tail of Acetes (?)
FIGURE 21.—Second antennae of Penaeus protozoea.
FIGURE 22.—Second antennae of nauplius of Penaeus.

Numbers 1–7 in figures refer to the number of the spines, counting from the inside.

LIFE HISTORY OF THALASSEMA. Abstract. By H. W. CONN, Assistant in Biology. With Plate III.

The observations embodied in this abstract were made at Beaufort during the summer of 1882. Hoping to be able to continue the observations and make them more complete, I reserve the publication of a full paper until some future time, and here give a brief abstract of some of the most important results.

Our knowledge of the development of the Echiuridae has been rather meagre. A paper by Hatschek,[1] a short account of Echiurus by Salensky,[2] and a note by Kowalevsky,[3] contain, as far I know, all the observations which have been made upon the subject. These papers are entirely confined to the later stages, and except that Kowalevsky stated that Thalassema passed through a regular segmentation, nothing of the early history has been known. My own observations, which are at present confined to the early stages, are supplementary to those already published, and fill up a gap which existed in our knowledge of the embryology of the group. With these observations we have a tolerably complete embryology of the Echiuridae.

The species of Thalassema found at Beaufort is an undescribed species to which I have given the name Thalassema mollita. It is of a dull red color, with a light yellow pre-oral lobe. A full grown adult reaches the length of an inch exclusive of the pre-oral lobe. The skin is nearly smooth, but is near the anus roughened by some whitish papillae. White bands, eight in number, extend from one end of the body to the other, marking the place of local muscular thickenings. The pre-oral lobe is long and flexible, and in the living animal is kept in constant motion. This species has contracted the habit of seizing upon empty sand-dollar shells and making its home in them. It

[1] Hatschek. Entwicklungsgeschichte von Echiurus. Arb. a. d. Zool. Inst. Wien, III.

[2] Salensky. Metamorphose des Echiurus, Morph. Jahr. II.

[3] Kowalevsky. Zeit. f. wiss. Zool. XXII, p. 284.

enters the shell at the oral opening while very small, but once within, it grows to considerable size, and remains the rest of its life a prisoner. One fact about this habitation, very convenient for the collector, is that each inhabited shell is marked in a peculiar manner. Directly over the animal is seen a reddish brown horseshoe-shaped mark, which makes it very conspicuous and enables one to select at a glance all the inhabited shells.

The anatomy of Thalassema is very much the same as that of Echiurus, described by Spengel.[1] Thalassema is dioecious. The ova and mother-cells of spermatozoa are simply modified cells of the peritoneal lining of the body cavity. Stretching from the wall of the body to the intestine are numerous muscular bands, one of which, situated at the posterior part of the body near the anus, marks the position of the sexual organs. The cells of the peritoneum covering this septum are converted into the sexual products. If this septum be examined during the breeding season it will be found covered with a large number of round cells which are rudimentary ova or mother-cells of spermatozoa, all of them very small. They do not go through their development in this position, however, but very soon break away from the septum and float freely in the body cavity. They have thus early, almost before they can be distinguished as ova, lost all connection with the body and are independent cells, floating to and fro in the peri-visceral fluid. From this fluid they absorb nutriment and rapidly grow. A large number of yolk-spherules make their appearance in the egg, the germinal vesicle becomes distinct, and the vitelline membrane makes its appearance. The mother-cells of spermatozoa divide into a large number of male cells, all of which, however, remain connected together in an irregular spherical mass. Having reached maturity the ova and spermatozoa masses are driven by some mechanism not well understood, into two pair of sexual pouches (homologues of segmental organs) situated at the anterior part of the body, a short distance behind the mouth. In these pouches they remain for an indefinite period. During the summer these sexual pouches are always found filled, or partly filled, with ova or spermatozoa, which are always sexually mature. They stay in this condition

[1] Spengel. Zeit. f. w. Zool. XXXIV.

for a long time, those first produced probably remaining in the pouches for many weeks before they are discharged. They are probably all discharged at nearly the same time through an opening by which each pouch communicates with the exterior. I have not, however, observed this discharge.

The segmentation presents a number of interesting points. As obtained from the sexual pouches, the ova are never spherical, owing to the fact that they are so closely packed together. As soon as fertilization takes place, however, they become perfectly spherical, and the germinal vesicle at the same time disappears. Unfortunately the multitude of yolk-granules prevents any observations on the internal phenomena accompanying segmentation.

In about fifteen minutes after fertilization the vitelline membrane is slightly elevated at one point, and a small, perfectly clear mass of protoplasm is protruded from the egg, the first polar globule. A second soon makes it appearance, and the first one divides into two parts. This seems to be the rule in all normally developing ova. One interesting fact was noted in the protrusion of these polar globules. There is exhibited the same rhythm of alternating periods of activity and rest that has been so many times observed in segmenting ova. The first period of activity (the protrusion of the first polar globule) was universally succeeded by a period of rest lasting ten minutes. The second period of activity was succeeded by a second rest, of very slightly longer duration. Then followed the segmentation with its rhythm, which seemed to be a direct continuation of that of the polar cells, except that the resting stages were slightly longer. An argument is here found for considering the protrusion of polar globules as a process homologous with segmentation. Three points were noticed in regard to these bodies. 1. The protrusion of the polar globules is brought about by fertilization, never occurring before fertilization, but appearing in a few minutes after. 2. It is a process to be compared with segmentation, as is shown by its possessing a like rhythm. 3. The polar globules are formed within the vitelline membrane, and are not, as is sometimes the case, protruded through it.

The segmentation is perfectly regular and uniform, a fact of peculiar interest. The work of Hatschek has shown that the Echiuridae are undoubtedly Annelids. Now all Annelids, with

perhaps the exception of Serpula and Turebellidis, have a segmentation more or less irregular. A reason for the peculiarity exhibited by Thalassema is found in the fact that the entire development takes place in the water. An egg which goes through all its changes in a homogeneous medium of nearly its own specific gravity will evidently not be acted on by gravity in such a manner as to bring about an accumulation of food-yolk at one pole of the egg. And since it is to such an accumulation that irregularities in segmentation are due, we find here a reason why the segmentation of Thalassema is regular. Not only is this true of Thalassema, but the same principle is found to be of considerable importance in other groups of the animal kingdom. For instance, Plathelminthes, Polyzoa, Chaetopoda, Discophora, Tracheata, Crustacea and Vertebrates, are groups in which is found an irregular segmentation, and at the same time have an ovum usually stationary and always protected in some way or other. On the other hand, the Echinodermata, Chaetognath and many Coelenterata have a freely floating ovum and a regular segmentation. This point cannot in this place be further expanded.

The result of segmentation is a spherical morula of ciliated cells containing a small segmentation cavity. About the sixth hour the second stage of development begins, the formation of a gastrula. This is formd by a typical invagination. At the same time two other important changes take place. 1. The region *opposite* the blastopore becomes marked off as the anterior extremity, and already functions as a head. It develops a tuft of sensory cilia, which are carried stiffly protruding and do not share in the motion of the general covering of cilia, and further it soon develops an ectodermal thickening, the future supra-oesophageal ganglion. 2. The larva which has hitherto simply exhibited a revolutionary motion, owing to its *uniform* coat of cilia, begins to assume a translatory motion with the anterior end in advance. This is due to the appearance of a band of long locomotor cilia around the blastopore, which future study proves to be the pre-oral band. It is thus possible even in this early period to distinguish an anterior from a posterior end, and to thus trace the relation of the gastrular axis to that of the adult.

The gastrula is now transformed into a trochosphere larva as follows: The invaginated sac, which has now grown quite large,

becomes divided into two parts, an oesophagus and a stomach. They both rapidly increase in length, and the digestive tract becoming longer than the body cavity (the original segmentation cavity) becomes doubled upon itself in such a way that the blind end lies near the mouth. Meanwhile the larva has elongated in a direction *not coinciding with the long axis of the gastrula*, but more nearly at *right angles to this axis.* There is thus a change in the long axis of the larva, which is now nearly at right angles to the original gastrula axis. The blind end of the digestive tract unites itself to the body wall at the extremity of the elongated larva, an opening breaks through forming the anus, and finally a constriction appears separating the stomach from a smaller intestine, thus completing the alimentary canal. Owing to this peculiar elongation of the larva, the relation of the trochosphere thus formed is different from what might have been expected. This relation can, however, be best understood by means of the accompanying figures, Fig. *A*, *B*, *C* and *D*, Plate III, than by any description. In these figures the line AB is the original gastrula axis, and the line CD is the axis of the trochosphere. This method of formation of the trochosphere, particularly the change in the direction of the long axis, forms a strong confirmation of Balfour's theory of larval forms as advanced in his Comparative Embryology. A comparison of Figures *A–D* with his figures (Fig. 231, Vol. II.) will show that what actually takes place in the Thalassema is just what he supposes to have taken place in the phylogenetic history of larval forms.

The ciliated band mentioned above as appearing very early becomes changed. At the gastrula stage and in the early trochosphere it consisted of a wide band of cilia, situated in the gastrula around the blastopore, and in the trochosphere in front of it, Figs. *A–D*. This band disappears and becomes replaced by a single row of very long, powerful cilia. This is the pre-oral ciliated band of Annelid larvae, and seems to be not the same structure as the ciliated band of the gastrula, though it has the same position and the same function. The new ring of cilia is a much more powerful locomotor organ than the old band, owing to the great length and power of the individual cilia. This is immediately made evident by the fact that the larvae now move with much greater freedom than before, swimming beneath the

surface rather than at the surface. The anterior region, which has by the growth of the larva become pushed to one side of the mouth, Figs. *A–D*, now becomes still more definitely designated as the head end by the greater development of the nervous ganglion and the uniformity with which this end is kept in advance.

The mesoderm appears very early in the history of the larva, and arises in two different ways, forming two different mesodermal systems. First, there is formed the two mesodermal bands so common to Annelid larvae, as has been so beautifully shown by Hatschek.[1] The second part of the mesoderm consists of a large number of unicellular muscles which extend from the alimentary canal to the body wall, especially at the region of the oesophagus. They regulate the movements of the oesophagus and stomach as well as slightly enlarging their cavities. These cells are originally separated off from the endoderm at the time of the invagination or somewhat later, having thus an origin very similar to that of the mesoderm in Echinoderms, as shown by Salenka.[2] There is thus present in Thalassema mesoderm, corresponding to both of Hertwig's[3] divisions, enterocoela and pseudocoela.

Three other ciliated bands soon make their appearance. One, a post-oral band, appears immediately behind the mouth, and like the pre-oral band, consists of a single row of cilia. A second band is developed just in front of the anus. A third belt of cilia is of more interest. It is found upon the ventral median line, *i. e.* the region between the mouth and the anus, in precisely the place where the ventral nerve-chain is to arise. It is thus seen that both the cerebral ganglion and the ventral nerve chain are preceded by the development of cilia from the very cells from which the nervous elements are to arise. It is an interesting point as indicating that already these cells are differentiated as nervous elements, although at first there is no trace of any nervous system. The pre-oral band probably has a similar meaning and may be homologous with the marginal nervous ring of medusae.

[1] Hatschek. Arb. a. d. Inst. d. Universitaet, Wien, 1878.
[2] Salenka. Zeit. f. wis. Zool. XXVII, 1876.
[3] Hertwig. Cœlomthorie. Jena, 1881.

The larva formed as above described is a typical trochosphere larva, agreeing in most respects with that of Echiurus and Annelids in general. I was unfortunately unable to keep my larvae more than seven weeks, when they were killed by a violent thunderstorm. Up to that time the only important further changes which had taken place were the appearance of a ciliated excretory(?) organ connected with the intestine, and opening into the body cavity; the *segmentation* of the mesodermal bands; and the development of the ventral nervous chain. The latter appear as two nerve chains, one on each side of the median line, almost precisely as is described in Lumbricus by Kleinenberg.[1]

The more important points observed and studied are therefore as follows:

1. The origin of the ova and spermatozoa as modified peritoneal cells, their growth in the body cavity, and their preservation in a sexually mature condition in the sexual pouches.
2. Protrusion of two polar globules exhibiting a rhythm precisely similar to that of the segmenting ova.
3. Segmentation, which is exceptionally among Annelids perfectly regular.
4. Formation of gastrula by a typical invagination.
5. The early appearance of a pre-oral band of cilia, and its subsequent disappearance and replacement by a *row* of longer, more powerful cilia.
6. The transformation of the gastrula into a trochosphere by a peculiar method of growth.
7. Origin of mesoderm as twofold, and the segmentation of mesodermal bands.
8. Origin of ventral nerve chord from the ectoderm as a bilateral structure.

Figs. *A–D* illustrate the change of the gastrula larva into a trochosphere.

[1] Kleinenberg. Development of Lumbricus trapezoides. Quar. J. Mic. Sci. XIX.

OF THE GILL IN SOME FORMS OF PROSOBRANCHIATE MOLLUSCA. By HENRY LESLIE OSBORN, Fellow by courtesy in Johns Hopkins University. With Plates IV, V, VI.

The researches of Peck, and later of Mitsukuri, upon the gills of the lamellibranchs have made it tolerably clear that the highly complicated gills of some of the lamellibranchs are derivable from a simple series of folds of the body wall. These results have an important bearing upon the question of the ancestry of this group, since they show that these ancestors had a far simpler gill than that of the lamellibranch of to-day. There are various reasons for supposing that this ancestry is to be found in the stock of the cephalophorous mollusca. At the suggestion of Dr. W. K. Brooks, I have undertaken to make a careful and minute study into the gills of the gastropoda, hoping that perhaps from this source additional light may be thrown upon the problem of molluscan phylogeny. It is with regret that I present this incomplete review of the prosobranch gill, and in the hope that I shall be able to secure the material for a thorough and complete survey of this entire question in the near future.

The gill of Chiton has been elucidated by Bela Haller, in the second part of his paper upon the Chitons of the Adriatic,[1] and he gives sufficient reason for considering the separate pouches along the mantle groove as individual gills and homologous with the gill of higher gastropoda. This view is accepted by Lankester in his article "Mollusca," in the Encyclopædia Britannica, and the homology was suggested by Dall and others before. The gill in the Adriatic species studied by Haller may be briefly described as a series of flat rounded plates attached above one another upon a central rachis, and bored through at opposite ends of the diameter of each plate by a vessel which enters upon the base of the gill on one side, runs up over the summit and down upon the other side. There are thus two vessels communicating with

[1] Haller, B. Die Organization der Chitonen der Adria. Claus, Arbeiten, Vol. V, 1883.

the blood supply, one of which is incurrent, the other excurrent. This is very similar to the plan of the gill in Fissurella, and is the only plan of the gill described for any Chiton.

In Chiton *sp. indet.*, which is abundant at Beaufort, North Carolina, upon shelly bottoms in protected "sounds," I found the gill to be unlike that ordinarily described for Chiton. It consisted of the usual circular flat lamellae arranged one above the other, and united by a central rachis, but with a single central bloodvessel running from the base to the tip of each gill, and without the two vessels for incurrent and excurrent blood flow. Fig. 1, *a*, *b*, *c*, is a series of sections in which the cavity of the blood-vessel is represented by dots, and the general lacunar tissue of the body cavity by parallel lines. From this it will be seen that the gill bears but a single blood-space and that this is central. Figs. 3 and 4 are from two sections, one to one side of the middle line, one in the middle line of the gill, and both longitudinal. There the epithelium is seen passing over from one plate upon the rachis to the next plate, leaving everywhere a narrow space for the exposure of a thin layer of blood to the surrounding water, except in the centre, where the large shaft is a blood reservoir. In a surface view of the gill which has been made transparent by immersion in turpentine (Fig. 2), this central shaft may be seen, though somewhat obscurely, through the other tissues.

In Fissurella *sp. indet.*, of which I have had but a single specimen for study, the gill is a paired organ consisting of a right and left body. Each is triangular in cross section, free from the surrounding tissues except at its origin, and tapering towards its free end, as shown in plan in Fig. 5. Along the apex of the triangle there runs a vessel carrying blood to the heart; opposite it upon the base of the triangle there runs a second vessel which carries blood from the heart into the gill. Between these two vessels there runs a string of tissue which separates the gill into two similar halves. Along this central string at right angles to it are placed the separate gill plates. Each plate (Fig. 6) presents the following parts: an apical portion *a* where the gill in its proximal portion is attached to the mantle; immediately under this the excurrent vessel (*v. ex.*). Upon either side of the excurrent vessel there is a section of the supporting framework (*r. f.*) of the gill flaps, which is strongly

thickened along the outer margin of each flap (*r*) and forms a thick U as it passes over upon the next gill flap. Running down the middle of the triangular plate is the central string of tissue the rachis, and at its end the incurrent bloodvessel (*v. in.*) A long series of such plates as this with the epithelium continuous form the gill of Fissurella. There is no union or fusion of the plates except at their bases where each one passes directly over into its successor. In Fig. 7 I have represented a section along the gill cutting through the excurrent vessel and the gill flaps; here may be seen the large central blood-space and its prolongation upon either side into the gill plates. A portion of one of these plates is shown more enlarged in Fig. 8. It consists of cuboid epithelium upon a heavy basement membrane which is strongly thickened at the outer margin (*r*) of the plate. The epithelium is strongly ciliated. Such epithelium as this is found generally in this situation; it will be shown further on that in their histology the greatest likeness prevails among all the gastropods studied.

It is a matter of great regret that I have not been able to get gills of a large number of Rhipidoglossa for study before proceeding to a study of the gill in the higher prosobranchs, but none were at hand, and their study must be left for some future occasion, when it is hoped to extend this study more widely than has as yet been possible. It would be especially interesting to see what has resulted from the degeneration of one gill in the scutibranchs, because the gill in all the higher forms of prosobranchs is very unlike that of Fissurella.

In Fulgur carica the gill forms a ridge running lengthwise in the roof of the mantle cavity upon the left side of the middle line. On its left side lies the so-called accessory gill, an organ shown by Spengel[1] to be an olfactory organ, and to function in testing the water given the gill for aeration of the blood. This gill is innervated from the right side of the body, and is the right gill, as is clearly shown in Spengel's paper; its position being accounted for by the spiral twist which has taken place in the portions of the body behind the cephalic region and above the foot. It is not free from the body even in the part farthest from the heart, but is united along its whole extent dorsally with

[1] Die Geruchsorgan der Mollusken, Zeitschr. f. w. Zool. Vol. 35.

the roof of the mantle cavity. It is composed of a series of triangular plates (Fig. 10), placed parallel to one another and vertical to the long axis of the gill. These plates are free from one another except in the roof of the mantle cavity, and they are united along their whole length to the roof of the mantle cavity. They are at first low, but grow higher till they present distinctly a deep flap hanging down into the cavity of the mantle. At its proximal end the gill is represented in Fig. 11. Here *Pl* is a surface view of the flap, while *a* is the ridge upon the body wall upon which it abuts. Running along the base of the gill in this part is a large blood-space. It is the excurrent vein and bears the blood from the gill to the heart. There is no definite incurrent vein, but the blood penetrates the lacunar tissues of the body with readiness, and passing among the spaces in the gill flaps is finally drawn off into the heart through the excurrent vein.

Transverse section through a series of gill plates is shown in Fig. 13. It is here seen that the gills are merely diverticula of the inner wall of the mantle; large folds in the basement membrane carrying with them the epithelium of the body wall and presenting this peculiarly modified for osmositic functions, and with differentiation in the basement membrane to make it a proper support for the epithelium. Each plate then presents three important parts, viz. central cavity, basement membrane and superficial epithelium. The central cavity (*g. cav.*) continuous with the body cavity is filled with blood, here and there outgrowths pass across this cavity to the wall opposite and form a stay to bind the two sides of the plate more firmly together. On either side of the gill cavity is the basement membrane, and this in most of the gill plates is very thin and delicate, but along the outer margin of the gill (*r*) it is very greatly thickened and forms a stiff rod of chitin to give additional strength at this outer portion. The epithelium upon the gill plates (Figs. 14, 14*a*) is of two sorts. Upon the outer free border it is composed of high columnar cells which stand compactly; upon the inner portion these columnar cells are replaced by cells much lower and placed very loosely. It seems probable that these inner cells are especially adapted to facilitate a rapid interchange of gases; and the outer cells, while respiratory, are better adapted to stand the rougher usage to which their exposed position would subject them. These cells

are entirely unlike those upon the remaining portion of the mantle (Fig. 12); those on the outside wall are lower and more cuboidal, while the inner mantle wall is thrown into wavy lines of basement membrane forming pockets which are lined with large mucous cells and presenting a characteristically glandular appearance. All the epithelium cells of the gill are very strongly ciliated, though the cilia of adjacent cells are often entangled and torn away, so that a bald, apparently non-ciliated cell results. These plates are further always entirely independent of one another, never united by those ciliated junctions and concrescences of tissue so common in the gill of the lamellibranch, binding the plates of the gill there into a compact organ.

The gill of Fulgur is quite similar to that which prevails among the higher Prosobranchs, the Ctenobranchia of Claus's classification.[1] The above description would apply perfectly to Fusus, Littorina, Nassa and Lunatia, all of which I have studied.

In Sigaretus *sp. indet.*, we have a close ally of Lunatia, whose gill is almost precisely like that of Fulgur; but in Sigaretus the gill is considerably modified. It occupies as in Fulgur the left side of the mantle-cavity, has the same nerve supply; and at its base runs the excurrent bloodvessel. But the individual plates have a different shape. They are of high triangular outline, and stand with the narrow base upon the mantle as shown in Fig. 15. A surface view of one plate shows an outer border traversed by a supporting rod, and distinct ribs upon the outer part of the plate; a distinct central line beyond which, upon the inner portion of the plate, the transversely ribbed appearance continues, but with ribs much farther apart. This appearance of ribs in the gill plate is due to two causes; in the outer portion the basement membrane is very strongly corrugated as shown in Figs. 16, 16*a*. The outline of the outer portion of the epithelium is not affected by this condition of the basement membrane, but remains smooth and regular. The corrugation further takes place in one direction only, the ridges lying transversely to the plate. Upon the inner portion of the gill plate the basement membrane is not corrugated, but the whole plate is thrown into a long series of transverse folds, as shown in section in Fig. 18, and this folding takes

[1] Grundz. der Zool., Vierte Auflage, II, p. 49.

place in both the basement membrane and the gill epithelium carried thereon. The basement membrane is strengthened to form the supporting rod along the outer border. One may further discover in surface view (Fig. 15*b*) circular spots, like the appearance in longitudinal sections of coniferous wood, lighter than the membrane generally, and these seem to be either thin places or openings, probably the former, to facilitate osmotic transfers.

An examination of the epithelium of the two portions of the gill shows the same difference to exist here as noted in the gill of Fulgur. Upon the outer portion (Fig. 17), the epithelium forms a compact, closely built wall (this section being transverse to the gill plate does not show the corrugation as does Fig. 16, a longitudinal section). The epithelium on the inner portion of the gill (Fig. 19) is composed of large loose cells of no very definite outline. They seem, if ciliated at all, to be very feebly so. I found no cells in any of my sections which I could positively affirm to be ciliated, though in many cases the appearances indicate the presence of small cilia.

In Crepidula fornicata (Fig. 20) the mantle cavity is broad and shallow, and the gill is composed of a series of fine threads which stretch across it from the left side toward the right. The filament arising from a ridge at the extreme left of the mantle cavity is united with the mantle during the first part of its course, but farther on it is free and stretches far out, like a finger (Fig 22). Each filament is composed of the basement membrane, bearing ciliated cells (Fig. 26), and with the basement membrane thickened strongly in the free portion to form a strong support upon which to suspend the delicate epithelium cells. At their bases (Fig. 27), the gill rods merely meet one another. They do not fuse, one passing directly over into the next, as they do in forms hitherto described (Fig. 15 *a*). The proximal portion of these filaments (Fig. 23) presents much the same condition as the ordinary forms of gill. The long filamentary outer part of the gill is represented in section in Fig. 25. At the proximal end, after the supporting rod has disappeared, the plates are fused at their outer ends (Fig. 24), and not in the centre, as though the space between each pair of gill plates ran into the body wall and there ended blindly. The cilia upon the gill filament-epithelium are everywhere strong and robust, though not represented in the drawings.

While these two forms of the ctenobranch gill, Crepidula and Sigaretus, are unlike Fulgur's gill, the divergence is plainly only a superficial one, and in Crepidula at least is very readily explained as an adaptation. Crepidula creeping over other shells has found that its very flattened body offers least resistance to forces tending to tear it away, and has acquired flatter and flatter shape. To permit this the mantle cavity has been broadened and flattened till there is but a very narrow space left for the accommodation of the gills, and the high plates have been modified into low and long three threads which offer a large surface but stow away better than the ordinary ctenobranch gill.

We may sum up the facts thus far stated in regard to prosobranch gills as follows: In Chiton and in Fissurella the gills are leafy blades attached to a rachis and joined to the body only for a short distance, the base of this rachis; in the prosobranchs generally the gill consists of independent plates, each one attached to the roof of the mantle cavity, and not placed upon a stalk and borne free from the mantle wall. The few forms which are divergent from this plan of structure are readily seen to be only secondarily different from it. Paucity of material has prevented the investigation of all the forms of Prosobranch gills, and this is very unfortunate, because there are among them some very divergent ones. Struthiolaria has a filamentary gill (Cuvier). Valvata is described and figured by Bronn with a gill superficially resembling Fissurella, a central stalk with gill plates borne free from the body of the animal, except at its proximal portion.

In the article Mollusca, in the last edition of Encyclopædia Britannica, Professor E. R. Lankester states his belief that the primitive gill of the Mollusca was a *ctenidium*, a stalk with plates very much like the gill of Chiton and Fissurella, and that the gills of all Mollusks are not homologous organs, but that some are independent structures, which have acquired a branchial function. He would thus consider the dorsal and anal gills of nudibranchs not ctenidia but secondary gills, while the gills of the cephalopods would not disagree with his view of them as *ctenidia*. The view, that the prosobranch gill is derived from some primitive form like Chiton gill, is pro-

posed by Spengel,[1] who thinks that gradual coalescence of the free distal portion with the mantle wall has given rise to the present form predominating among the prosobranchs.

Are we to accept this view of Lankester and to consider the gill as we find it in most ctenobranchs derived from a *ctenidium* by modification, or shall we regard the common form of ctenobranch gill as the most primitive? The embryonic history of the ctenobranch gill is the same throughout. It arises as a series of folds in the wall of the mantle or independent leaves or pouches in Crepidula. In this latter case the pouches extend across the mantle cavity roof like fingers of a hand (*vid.* Fig. 29). In Eurosalpinx and other forms the gills are plates which grow broader and longer, and are eventually strengthened with supporting tissue. A transverse section across the animal (Fig. 31) shows these plates to be foldings in the inner wall of the mantle, and the later growth in the structure of the gill is merely a stiffening and strengthening of the basement membrane, upon which the epithelium is placed.

We do not see any resemblance in this embryonic history to the ctenidium of Lankester's Protomollusk.

The researches of Peck and Mitsukuri have rendered it tolerably clear that the highly complicated lamellibranch gill owes its complexity to secondary causes, the sessile habit of the animal throwing upon the gills sundry functions besides those of the gill proper, and it seems quite certain that in these forms the gill is to be considered as primitively "a simple ridge on the side of the body,[2] with folds on two sides of it."

It is this in the young Mytilus which I have observed, where the gills, a series of folds of the mantle, look almost precisely as in the young of many prosobranchs.

The opinion is growing among zoologists that the Lamellibranchs are not to be considered as a group preceding the gastropoda in the phylogeny, but that they are forms derived from cephalophorous ancestors, perhaps gastropods or their immediate progenitors, and that the loss of head and lingual ribbon is

[1] Die Geruchsorgan und das Nervensystem der Mollusken. Zeitschr. f. w. Zool. Vol. 35, p. 355.

[2] Mitsukuri. Biol. Studies, Johns Hopk. Univ., Vol. II. p. 268.

due to degradation rather than indicating a greater simplicity of structure.

Now this common form of ctenobranch gill is precisely similar to the form of lamellibranch gill considered most primitive. But whereas the lamellibranch by its adaptation to conditions unnatural for its ancestors has now a widely divergent gill, the ctenobranch gastropod, still retaining the ancient habits quite perfectly, has retained its gill with but, for the most part, little change.

In view of these considerations it does not seem to me safe to adopt the view of Lankester that the *ctenidium* is the primitive form of molluscan gill. To be sure it is the form most like the gill of Chiton, apparently a very primitive mollusk, and is found in Fissurella and its close allies. It is not quite safe to pronounce against this from the study of a few of the ctenobranchs and to assert that the present ctenobranch gill is but slightly modified from the ancestral type, but the known facts respecting the ctenobranch gill certainly do not readily harmonize with its derivation from a *ctenidium.* The extreme antiquity of the group would furnish time for all traces of the intermediate steps to have been obliterated, and hence it might be urged on the other hand that the *ctenidium*, while not characteristic of the higher prosobranchs, is lost in them, but retained in the Rhipidoglossa as well as in the whole group of cephalopods. It would only be possible to definitely settle this question by a more complete knowledge of the molluscan gill than is at present possessed.

We may briefly summarize the results of the foregoing studies as follows: The gill of Chiton and Fissurella is closely like the *ctenidium* which Lankester considers the primitive type of molluscan gill. In ctenobranchs, almost universally, the gill is not a *ctenidium*, but a very much simpler organ. Its form compares closely with the gill which we have come to regard as the primitive lamellibranch gill. Incomplete study of ctenobranchs and ignorance of the history of the development of the ctenidia in Chiton and Fissurella prevent more than a conjectural conclusion. It does not seem, however, safe to accept the conclusions of Spengel and Lankester that the ctenobranch gill is derived from a feather-form gill like that of Fissurella by the fusion of one side with the body wall.

LIST OF FIGURES.

CHITON.

PLATE IV.—FIGURE 1, *a*, *b*, *c*, series of sections along the pallial groove, showing the relations between the blood-space and the gill plates.

FIGURE 2.—One gill, surface view.

FIGURE 3.—Longitudinal section, one side of the centre magnified 235 diam.

FIGURE 4.—Longitudinal section through centre, × 175 diam.

FISSURELLA.

FIGURE 5.—Plan of the gill.

FIGURE 6.—Surface view of a single plate.

FIGURE 7.—Longitudinal section transverse to the plates and passing through the excurrent vein.

FIGURE 8.—Enlarged view of the outer portion of a single plate.

FUSUS.

FIGURE 9.—Animal (after Bronn) with mantle cavity laid open, showing position of gill and olfactory organ.

FULGUR CARICA.

FIGURE 10.—Plan of gill plate, showing outer mantle wall with the independent plates hanging from it.

PLATE V.—FIGURE 11. Surface vein of the proximal portion of single gill plate, × 20.

FIGURE 12.—Glandular epithelium of the mantle beyond the gill, × 175 diam.

FIGURE 13.—Transverse section of several successive plates, × 175 diam.

NEVERITA DUPLICATA.

FIGURES 14 and 14 *a*.—Epithelium outer and inner portions of gill plate, × 218 diam.

SIGARETUS.

FIGURE 15.—Surface view of a single plate.

FIGURE 15*a*.—Section along the ribbed basement membrane

FIGURE 15*b*.—Surface view of small portion of the basement membrane, with the epithelium removed, showing circular pits.

FIGURE 16.—Longitudinal section of a single plate, × 50 diam.

FIGURE 17.—Transverse section of a single plate, outer portion, × 218 diam.

FIGURE 18.—Longitudinal section of the inner portion, × 218 diam.

FIGURE 19.—Epithelium from the inner portion, × 175 diam.

CREPIDULA FORNICATA.

PLATE VI.—FIGURE 20. Animal shell removed; seen from dorsal surface, × 3.

FIGURE 21.—Portion of the roof of the mantle cavity, cut out along dotted line of Fig. 20; showing the gill filaments.

FIGURE 22.—Gill filaments enlarged, × 12.

FIGURE 23.—Transverse section of gill, inner attached portion, × 80.

FIGURE 24.—Transverse section of gill, proximal portion, × 80.

FIGURE 25.—Transverse section of gill, free outer portion, × 80.

FIGURE 26.—Transverse section of a single filament, × 175.

FIGURE 27.—Horizontal section at the base of gill.

FIGURE 28.—Plan of gill.

FIGURE 29.—Young Crepidula.

FIGURE 30.—Young Eurosalpinx (?)

FIGURE 31.—Section of young Eurosalpinx in line *ab*.

ABBREVIATIONS.

a.	Attachment of gill to mantle.
V. ex.	Excurrent vein.
V. in.	Incurrent vein.
r. b.	Chitinous rod at basal part.
r.	Chitinous rod in gill plate.
Pl.	Gill plate.
Nv.	Gill nerve.
ol.	Olfactory organ—"*accessory gill.*"
w. b.	Body wall next to shell.
w. r.	Body wall toward mantle cavity.
b. cav.	Body cavity.
g. cav.	Gill cavity.
m. o. s.	Outer surface of mantle.
[illegible] *s.*	Inner surface of mantle.

Col. m. Collumellar muscle.
sh. Shell decalcified, and appearing as a membrane about the young.
ec. Outer body wall.
Per. Pericardium.
Ph. Pharynx.
m. cav. Mantle cavity.
f. Foot.

NOTES ON THE COMPOSITION OF THE BLOOD AND LYMPH OF THE SLIDER TERRAPIN (*Pseudemys rugosa*). By WM. H. HOWELL, PH.D., Fellow of the Johns Hopkins University.

This investigation of the blood and lymph of the terrapin was suggested by a paper of Tiegel's on snake's blood (1). According to Tiegel, blood taken from the aorta of a snake (*Elaphis* and *Tropidonotus*) does not usually coagulate in less than 3½ hours, and sometimes not even after 24 hours; while blood from the inferior vena cava above the liver coagulates within fifteen minutes. If the inferior vena cava is ligated, and the venous blood taken from the sinus venosus, it does not coagulate any more quickly than that from the aorta. He concludes, therefore, that the rapid coagulation of the inferior cava blood is owing to something that it receives from the liver. These facts, if correct, seemed to demand a more thorough investigation, and snakes not being obtainable during the winter months, a number of experiments were made upon terrapins (*Pseudemys rugosa*) to determine whether the blood of this animal shows the same peculiarities. The results of these experiments show that such is not the case.

Terrapin's blood, taken from the left aorta or the pulmonary artery by means of a cannula, and received into carefully cleaned watch-crystals, requires usually from one to four hours for complete coagulation; that is, the formation of such a firm clot that the crystal can be inverted without losing any of the blood. In some cases the arterial blood coagulated in less than an hour; in other cases, when the corpuscles had settled more quickly than usual, the supernatant plasma remained perfectly fluid, if not disturbed, even after twenty-four hours.

Blood taken from the inferior cava or the hepatic vein by means of a cannula was found, at first, to clot in from ten to thirty minutes, but it was discovered afterwards that this was caused by an admixture of lymph, which in this region is very

abundant. In collecting blood from the inferior cava by means of a cannula it was necessary to make the cannula as short as possible, on account of the extremely slow flow, and unless special precautions were taken some of the lymph or pericardial liquid was likely to get mixed with the blood. When the venous blood was obtained by ligating the vein, cutting a small hole in its walls and then removing the blood by means of a pipette introduced directly into the vein, this difference in the time necessary for coagulation was not observed. Blood taken from the aorta, inferior cava, hepatic vein and superior cava showed no constant difference in the time of coagulation. Sometimes the venous blood coagulated first, sometimes the aortic blood. As a rule the venous blood coagulated more quickly, but the difference in time was never very great.

As stated above, it was found that the blood, whether arterial or venous, when mixed with an equal bulk of pericardial or pleuro-peritoneal liquid, coagulated rapidly, requiring only from ten to twenty minutes. Quite small quantities of these liquids have a marked influence in hastening coagulation. This action is doubtless dependent on their abundant water breaking up the white corpuscles and setting free the fibrin ferment. Distilled water was found to have an exactly similar effect; indeed, coagulation was usually more rapid with it than with the animal's lymph, since the strong alkaline reaction of the latter is unfavorable to the formation of fibrin.

I have not had an opportunity of making any experiments upon snakes, and cannot say whether the more rapid coagulation of the venous blood in them is also caused by an admixture of lymph, though I think it probable that this is the true explanation of Tiegel's observations.

I.—*The Composition of the Blood of the Terrapin.*

The time necessary for the coagulation of terrapin's blood varies greatly in different individuals. In some cases, the blood, when kept in shallow layers in wide dishes, coagulates completely within an hour; at other times coagulation is very much slower. In some specimens of blood the corpuscles settle quickly to the bottom and form an imperfect clot, while the clear supernatant plasma may remain uncoagulated for twenty-four hours or longer.

If it is desired to get serum, this settling of the corpuscles must be prevented by gently agitating the blood from time to time; the clot formed from the corpuscle-free plasma presses out but little serum.

The quantity of blood which can be obtained from a single terrapin of medium size varies from 50 cc. to 125 cc.; that is, for animals obtained during their winter fast, as was the case with most of those used in my experiments. The blood possesses a decided alkaline reaction when first drawn. When it is allowed to clot in shallow vessels, and the clot formed is gently separated from the walls of the vessel, it yields a large quantity of clear serum, with a strong alkaline reaction. The alkaline reaction is so strong that in some cases the serum, when poured into a quantity of boiling water, gave only a faint opalescence and no precipitate.

A number of analyses were made to determine the quantities of paraglobulin and serum-albumen contained in the serum. The method of analysis was to determine first the total proteids in 5 cc. of serum, and then the paraglobulin in an equal quantity of the same serum, the difference between the two giving the serum-albumen.

To find the total proteids, 5 cc. of serum were dropped into 50 cc. of boiling water, and dilute acetic acid, 1 per cent., carefully added until the precipitate settled from a perfectly clear liquid. The precipitate after cooling was collected on a filter previously heated to constant weight at 110° C., washed thoroughly with water and boiling alcohol, and then heated to constant weight at 110° C.

For the paraglobulin Hammarsten's method of saturation with $MgSO_4$ was used. 5 cc. of the serum were mixed with 25 cc. of a saturated solution of $MgSO_4$, and then finely powdered $MgSO_4$ added until complete saturation was effected. The solution was allowed to stand for twenty-four hours, filtered through a weighed filter, and the precipitate thoroughly washed with a saturated solution of $MgSO_4$ until the filtrate no longer gave an opalescence on boiling. The filter and precipitate were heated to 110° C. for several hours, washed with boiling water until the $MgSO_4$ was completely removed, then with boiling alcohol, and finally heated to 110° C. until the weight became constant.

The following results were obtained from animals in a fasting condition, the stomach and intestines being completely empty. The analyses were made usually with 5 cc. of serum, but the numbers are given for 100 cc.

No. I.

Total proteids = 5.701 grms.
Paraglobulin = 4.697 "

Serum-albumen = 1.004 " or 17.6 per cent. of the total proteids.

No. II.

Total proteids = 5.379 grms.
Paraglobulin = 4.777 "

Serum-albumen = 0.602 " or 11.1 per cent. of the total proteids.

No. III.

Total proteids = 5.082 grms.
Paraglobulin = 4.510 "

Serum-albumen = 0.572 " or 11.2 per cent. of the total proteids.

No. IV. Analysis of the corpuscle-free plasma.

Total proteids = 3.938 grms.
Paraglobulin + fibrinogen = 3.174 "
Fibrin = .3745 "
Serum-albumen = .764 or 19.4 per cent. of the total proteids.

The most striking result of these analyses is the small amount of serum-albumen present in the blood. Since the terrapins had been without food for a long time, and the stomach and intestines were completely empty, it was thought that perhaps this was the cause of the small percentage of serum-albumen. Tiegel states that in the blood of starved snakes, after diluting the plasma with water and precipitating the paraglobulin by CO_2, he could detect no serum-albumen by boiling the liquid; while in the blood of those snakes whose stomachs were found filled with food, serum-albumen was present. There seems to be some error about his first

statement, since in the blood of other animals the paraglobulin is not completely precipitated by CO_2, and the filtrate from his precipitate should have given a cloudiness at least, upon boiling, due to the paraglobulin still in solution, even if no serum-albumen was present.

Burckhardt (2), from experiments made upon dogs which had been starved for five or six days, found that while the proportion of paraglobulin increased (the increase varying from 22.8 per cent. to 66.4 per cent. of the quantity present before starvation), the proportion of serum-albumen suffered a marked diminution, varying from 5.3 per cent. to 21.66 per cent. of the quantity present before starvation. On the other hand Salvioli (3), from experiments also made upon dogs, found that there is no actual difference in the relative amounts of globulin and albumen present in the blood during starvation and a well-fed condition.

The difference in the results of these two investigators appears to be dependent upon the method used for the determination of paraglobulin. Salvioli made use of Hammarsten's method of precipitating the globulins by complete saturation with $MgSO_4$. Burckhardt used the older method of Schmidt, precipitating the globulins by dialysis. He states that Hammarsten's method is not reliable, and that the larger portion of the $MgSO_4$ precipitate resembles the globulins only in its precipitation by $MgSO_4$, while in its other reactions it acts like an albumen. He gives, however, no satisfactory proof of these statements.

Hammarsten's method, as far as our present knowledge of the proteids of blood-serum goes, is certainly the most reliable that has been offered. He has given many proofs himself (4) that by it none of the serum-albumen is precipitated along with the paraglobulin, the strongest of which, perhaps, is that in a concentrated solution containing as much as 11.8 per cent. of serum-albumen, no precipitate at all is produced by $MgSO_4$ added to saturation. Fredericq (5) has given independent proof of the correctness of the method, in that he has found that the substance precipitated by $MgSO_4$ has a rotation of $-47.8°$, while that left in solution has a rotation of $-57.3°$.

I have made several experiments upon terrapins in a state of digestion to determine whether the quantity of serum-albumen differs from that in the fasting condition, using Hammarsten's

method for estimating the paraglobulin. In two cases the animal was fed artificially with soup and pieces of fish; in one of these the food was completely digested after ten days, and the stomach glands examined under the microscope were found in full digesting condition. In another case a terrapin which was received in early spring, after it had recovered from its winter sleep and had begun to feed, when killed had its stomach filled with food in a state of good digestion. The serum of these last two terrapins when analysed by the method given above yielded the following results. The numbers are given for 100 cc. of serum.

No. V. Terrapin fed artificially.
Total proteids = 4.113 grms.
Paraglobulin = 3.693 "

Serum-albumen = 0.420 " or 10.21 per cent. of the total proteids.

No. VI. Terrapin found with full stomach when killed.
Total proteids = 4.002 grms.
Paraglobulin = 3.181 "

Serum-albumen = .821 " or 20.51 per cent. of the total proteids.

The two analyses certainly do not give results which agree well with each other, but the comparatively large amount of serum-albumen found in the last analysis is only 3 per cent. greater than that found in analysis I. of the serum of a fasting terrapin. So that the evidence goes to show that feeding increases but slightly, if at all, the quantity of serum-albumen present; and in either case the quantity of serum-albumen in the blood, compared with that of most other animals whose blood has been chemically examined, is unusually small.

II.—*Properties of the Paraglobulin.*

By paraglobulin is meant all of the proteid precipitated from serum by complete saturation with $MgSO_4$. The behavior of this precipitate when dissolved and heated indicates that it is probably composed of more than one globulin. It contains, at

least, one globulin not hitherto described, as will be shown later, formed from the fibrinogen during coagulation.

Judging from one experiment, Analysis IV, the quantity of paraglobulin present in the plasma is markedly less than that in the serum, as we should expect if paraglobulin forms one of the products of the breaking up of the white corpuscles. In that analysis, .3745 grm. of fibrin was found for 100 cc. of plasma; supposing that this represents 80 per cent. of the original fibrinogen, which is a liberal estimate, .4681 grm. of fibrinogen was present in 100 cc. of the plasma, leaving 2.7 grms. of paraglobulin, which equals 68.77 per cent. of the total proteids; while the analyses of serum show that in it the paraglobulin forms from 80 per cent. to 90 per cent. of the total proteids. When the serum is strongly diluted, and CO_2 is passed through the liquid, or a few drops of dilute acetic acid are added, a precipitate of paraglobulin is obtained as in the serum of other animals. This precipitate, however, when compared with that obtained from the same serum by saturation with $MgSO_4$, is much less in quantity.

Dialysis also causes a large precipitate. Two analyses of the paraglobulin were attempted by this method, to see how the results compared with those obtained by the $MgSO_4$ method. 10 cc. of serum were taken in each case and dialysed for 48 hours; the outer vessel contained three litres of distilled water, which was frequently renewed. After the completion of the dialysis the precipitate was washed from the parchment paper, diluted to 150 cc. with distilled water, and allowed to stand in the cold until the precipitate had settled to the bottom. The precipitate was then filtered (the filtrate gave no precipitate at all with CO_2) and washed in the usual way with water and alcohol. In washing the precipitate the liquid came through somewhat opalescent, so that some of the paraglobulin was lost by the process. The analyses, however, though on this account not perfectly correct, show, nevertheless, the incompleteness of this method when compared with the $MgSO_4$ method.

The following results were obtained for 100 cc. of serum:

Serum No. I, 0.8705 grm. of paraglobulin.
Serum No. II, 1.285 grm. of paraglobulin.

Complete saturation of the serum with NaCl throws down a very small precipitate, less than CO_2 or acetic acid, though no analyses were made to determine the exact amounts. Fresh alkaline serum when heated or thrown into boiling water gives often only a more or less strongly marked opalescence; in other cases, especially if the serum has been kept for some time, a strong precipitate is produced.

Many specimens of serum were neutralized or made feebly acid with acetic acid, and then heated in a test tube immersed in a double water-bath made from two beakers. Usually the serum became opalescent between 60° and 65° C., and between 65° and 68° C. gave a very strong precipitate. The precipitate sometimes formed at a lower temperature, depending apparently on the degree of acidity. The filtrate from the precipitate gave in most cases another small precipitate or cloudiness at 75° C., and the filtrate from this, when heated above 80° C. or boiled, gave a third slight opalescence or precipitate.

When the precipitate obtained from dilute serum by CO_2, or acetic acid, was dissolved in dilute NaCl solution and heated in the same way, an opalescence began to appear at 68°–70° C.; but a distinct precipitate first formed above 70° C., usually at 75° C.

A somewhat different result was obtained with the $MgSO_4$ precipitate. If this was freed from serum-albumen by repeated precipitations, or by washing with a saturated solution of $MgSO_4$, then pressed, dissolved in water and heated, evidence was given in most cases of the presence of two globulins. The first and by far the more abundant coagulated at temperatures varying from 68° to 73° C.; the filtrate from this precipitate gave a second small precipitate or opalescence between 75° and 80° C. This latter body, I think, is the globulin formed from fibrinogen during coagulation, since it coagulates at the same temperature.

The general result of the experiments shows that the coagulation temperature of paraglobulin lies between 68° and 75° C., or in most cases between 70° and 75° C. The temperature at which a precipitate is formed depends very much on the rapidity with which the liquid is heated. If heated very slowly or kept at a comparatively low temperature for some time, a precipitate may often be obtained which in more rapid heating would first appear at a temperature several degrees higher.

III.—*Properties of the Serum-Albumen.*

In order to determine the coagulating temperature of the serum-albumen, serum was completely saturated with $MgSO_4$, and, after standing 24 to 48 hours, filtered from the precipitate of paraglobulin; the filtrate was dialysed to get rid of the $MgSO_4$. The solution became highly diluted by this process, and attempts to concentrate it by evaporation at a low temperature were not successful. Exposure for several hours to a temperature of 45°–50° C. caused a partial precipitation, a fact observed also by Heynsius (6) for the albumen of mammalian serum. When the dialysed liquid, containing still a small percentage of salts, was slowly heated in the apparatus described, a more or less marked cloudiness appeared at about 80° C. This cloudiness sometimes began to be visible as low as 77°–78° C., and in several cases a distinct precipitate formed between 80° and 85° C.

The albumen of mammalian blood is said to coagulate at about 70° C., or between 70° and 75° C., at a temperature, therefore, somewhat lower than paraglobulin; the albumen of the terrapin's blood appears to differ in this respect, but the difference may depend upon the method of preparation, since Schmidt and Aronstein found that if the dialysis of the serum-albumen is carried far enough a solution is obtained which does not coagulate at all on boiling. This statement, however, has been denied by Heynsius and others, and cannot be accepted as an explanation of the high coagulating temperature of the albumen solutions obtained by me, since my dialyses were never made complete, a small percentage of $MgSO_4$ being allowed to remain in the solution.

IV.—*Properties of the Fibrinogen.*

The fibrinogen of terrapin's blood, when isolated by Hammarsten's method (see following article), differs markedly from the fibrinogen of mammalian blood as described by Hammarsten, in that it is completely precipitated by heating to 56°–60° C. The precipitate obtained at this temperature usually appeared as a fine opalescence, but when filtered the filtrate came through clear and gave no further precipitate or opalescence when boiled, and no proteid reaction when tested with HNO_3 and NH_4OH. In some cases, however, the filtrate did give a very faint opalescence on boiling, so slight that it could be disregarded.

It would appear from this that Fredericq's method of estimating fibrinogen, viz., by heating the liquid containing it to 60° C., would be perfectly reliable for the terrapin. When pure solutions of fibrinogen are used the method gives, of course, good results; but it is not applicable directly to the fibrinogen contained in plasma, since the alkaline reaction of the plasma prevents the complete precipitation of the fibrinogen at 60° C., and if the plasma is made neutral or feebly acid, some of the paraglobulin also may separate at this temperature.

When a pure solution of fibrinogen is mixed with ferment (see succeeding article) the fibrinogen is not completely converted into fibrin. A quantitative estimation in one case gave the following results. The solution of fibrinogen used was not very strong:

Fibrinogen in 100 cc. of the solution = .088 grm.
Fibrin from 100 cc. of the solution = .061 "

so that only 69 per cent. of the fibrinogen was changed into fibrin, a number which agrees with the determinations made by Hammarsten for the mammal. The whole of the fibrinogen, however, disappears in consequence of the action of the ferment; that portion not changed to fibrin becomes converted into a new globulin, coagulating between 75° and 80° C. The fibrinogen is completely precipitated from its solutions by saturation with NaCl.

No reliable method exists for determining the amount of fibrinogen in plasma. The most trustworthy, perhaps, is to determine the fibrin formed during coagulation, and from it to calculate the amount of fibrinogen present; but inasmuch as the percentage of fibrinogen converted into fibrin varies considerably, the results obtained by this method are only approximate. In Analysis IV the amount of fibrin formed was, reckoned for 100 cc. of plasma, .3745 grm.; considering this as 80 per cent. of the total fibrinogen present, we get for 100 cc. .4681 grm. of fibrinogen, which equals 11.8 per cent. of the total proteids.

That the fibrinogen exists in the plasma and not in the corpuscles is well shown by the observation recorded in the following article, in which a clear plasma was obtained which did not coagulate at all when left to itself, but gave a clot very readily

when mixed with a little clear serum. The inability to clot by itself showing the absence of ferment, and that none of the white corpuscles, or at least no appreciable number of them, had broken down, so that the fibrinogen present must have existed already in the blood-plasma.

The clear plasma obtained by allowing the corpuscles of the blood to settle in the cold for a day or two, usually contained sufficient ferment to cause it to clot in a short time when placed in a room at the ordinary temperature. When this plasma was filtered in the cold and then heated to 50°–52° C. for five minutes, its power of coagulating was completely destroyed. If the plasma was taken from the bottom layers containing a large number of white corpuscles and heated to the same temperature for five minutes, without previous filtration, coagulation, if it occurred at all, took place with extreme slowness; nevertheless when diluted with an equal bulk of water a clot was formed in a few minutes—the explanation apparently being that the white corpuscles resisted the action of heat, and when broken down by the subsequent addition of water yielded sufficient ferment to bring about coagulation. The action of the heat was probably two-fold, changing both the ferment and the fibrinogen. While aqueous solutions of ferment, as is shown in the following paper, are not perceptibly affected by heating to 50° C., for five minutes, yet when heated in the alkaline serum or plasma the action of the ferment is either very much weakened or entirely destroyed. On the other hand it was found that a solution of pure fibrinogen, when heated to 50° C. for five minutes and then mixed with ferment, gave no clot; while an aqueous solution of ferment heated to 50° C. for the same time and mixed with pure fibrinogen gave a clot in a few minutes.

V.—*The Pleuro-peritoneal Liquid of the Terrapin.*

The terrapin has an unusually large number of lymph sacs scattered through its body and filled with a very clear watery lymph. The abdominal cavity contains a large quantity of this lymph-like liquid, though in a strict sense of the word the name lymph can scarcely be applied to it, since its composition, and probably its origin, differ from those of lymph as usually understood. This abdominal liquid, in which the viscera are bathed,

exists often in extremely large quantities, sometimes exceeding the total quantity of blood in the animal's body. In some terrapins, however, under apparently the same conditions, very little of it was found. In the terrapins received at the beginning of winter, when hibernation had begun, and in those at the end of the season when the animals had again commenced to feed, the liquid was usually perfectly limpid, with some granular masses floating in it, which microscopic examination showed to consist of white corpuscles. These corpuscles exist in comparatively small numbers, and most probably do not properly belong to the liquid, but have wandered into it from neighboring bloodvessels. They can easily be filtered off. In other cases, especially in terrapins received in mid-winter and kept for some time in a moderately warm room, the liquid was colored a more or less deep yellow, and in some cases, evidently pathological, was decidedly bloody.

The normal pleuro-peritoneal liquid possesses a marked alkaline reaction, owing probably to the presence of alkaline carbonates, since neutralization with acetic acid is accompanied with effervescence. In the great majority of cases it showed no trace whatever of coagulation, even when kept at 30° C. for many hours. In rare instances I have seen specimens of the clear liquid which gave a feeble clot. Those specimens which were bloody usually gave an imperfect clot. Qualitative analyses of the clear liquid showed that the proteid present is almost entirely paraglobulin, with traces of serum-albumen and perhaps fibrinogen. To test whether any fibrinogen was present the liquids were neutralized with acetic acid and heated slowly to 60° C. In some cases not the slightest opalescence occurred at this temperature, in other cases a more or less deep opalescence made its appearance, especially if the liquid was kept at 60° for several minutes. This opalescence was caused doubtless in the majority of cases by the paraglobulin, since this proteid in neutralized serum often gives an opalescence at 60° C. In some few instances I have obtained evidence of the presence of fibrinogen, but, as a rule, if present at all it exists in very minute quantity. The addition of serum or an aqueous solution of ferment in a number of cases gave no clot, showing the absence of any appreciable quantity of fibrinogen.

When the liquid was saturated with $MgSO_4$ a considerable precipitate was always obtained. After standing twenty-four hours the filtrate from this precipitate gave sometimes no cloudiness on boiling, at other times a marked opalescence or precipitate, showing the presence of some serum-albumen. Quantitative analyses were made of two specimens of the clear filtered liquid, using the method described for blood-serum. The results were as follows:

I. Total proteids in 5 cc. = .0091 grm.
   Paraglobulin in 5 cc. = .0102 "[1]
II. Total proteids in 5 cc. = .0196 "
   Paraglobulin in 5 cc. = .0191 "

The clear liquids contain then practically no proteid except paraglobulin.

The alkaline liquid may be heated to boiling without giving any precipitate, and sometimes without showing any opalescence. If it is neutralized, or made feebly acid with acetic acid and heated, the most frequent result is an opalescence at 60° C., which separates out as a well-marked precipitate at 65° to 68° C., and a second smaller precipitate or opalescence at about 75° C. In some cases no distinct precipitate, even when the liquid was made feebly acid, was obtained below 75° C. The precipitate obtained by saturation with $MgSO_4$ gave the same reactions upon heating as that from the serum. Complete saturation with NaCl throws down only a small portion of the globulin. The paraglobulin, like that of serum, may be partially precipitated by careful addition of dilute acetic acid.

Although the clear liquids contain a number of white corpuscles, I was not able, for the few times that I tried it, to obtain a solution of ferment from them by Schmidt's method, except in one case; in this instance the precipitate produced by alcohol, after standing for three weeks, was extracted for a few minutes with water, and a solution obtained which gave no sign of a precipitate upon boiling and no proteid reaction with HNO_3 and NH_4OH, but which when mixed with a solution of fibrinogen gave a small clot. Not much importance, however, can be placed upon this single experiment.

[1] The error in this analysis was evidently caused by a failure to wash out completely all the $MgSO_4$.

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THE ORIGIN OF THE FIBRIN FORMED IN THE COAGULATION OF BLOOD.

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Of the different views which have been held with regard to the origin of the fibrin formed in the coagulation of blood, only two at present are taught in the text-books of physiology.

The older of these two views, and the one, perhaps, the more generally received, is that of Alexander Schmidt. According to the theory of Schmidt there are three substances, in addition to a certain quantity of neutral salts, which are necessary for the formation of fibrin, viz., fibrinogen, fibrinoplastin, or, to use a less objectionable name, paraglobulin, and fibrin ferment. The nature of the reaction amongst these three bodies, as usually given, is that the fibrinogen and paraglobulin interact or undergo a chemical combination of some kind under the influence of the fibrin ferment. Schmidt himself, after his discovery of the ferment, did not advance any theory of the chemical reaction that takes place, and expressly says that he is unable to offer any suggestion with regard to the nature of the decompositions or combinations that may occur. He contents himself with stating that the fibrin formed is the result of a fermentative process, the substratum of which is furnished by the fibrinogen and paraglobulin (1). The point that he insists upon is that both of these bodies are essential for the formation of fibrin. Fibrin can not be formed from either of them alone under the influence of the ferment.

The second of the two theories, that of Hammarsten, was first proposed by him about ten years ago, and from that time up to a year ago he has published numerous investigations in which the nature of the three bodies in question and their relations to fibrin have been fully discussed (2). According to Hammarsten, fibrin is formed from fibrinogen alone under the influence of ferment. A solution of pure fibrinogen prepared according to a method which will be described later, and containing no

paraglobulin whatever, gives when mixed with a solution of ferment likewise free from paraglobulin, a firm clot, yielding perfectly typical fibrin. The fibrinogen alone, according to this view, undergoes a process of fermentation resulting in the formation of fibrin.

The fibrinogen, however, is not completely converted into fibrin. The amount of fibrin obtained from equal quantities of fibrinogen varies greatly under different conditions; usually only from 60 per cent. to 80 per cent. of the fibrinogen is changed into fibrin. That portion of the fibrinogen molecule which is not converted into fibrin undergoes a further action of some sort, resulting in the formation of a new globulin with a coagulation temperature, 64°–66° C., intermediate between that of fibrinogen and of paraglobulin. Hammarsten has succeeded in isolating this body from blood-serum by partial precipitations with NaCl. Like fibrinogen it is completely precipitated from its solutions by saturation with NaCl, and resembles closely in elementary composition a globulin formed, according to Hammarsten, from a portion of the fibrinogen molecule when a solution of fibrinogen is heated to 56°–60° C. At this temperature the larger portion of the fibrinogen (from 60 per cent. to 90 per cent.) is coagulated, while a portion remains in solution as a globulin coagulating at 64°–66° C. He concludes, therefore, that the chemical process in the two cases, coagulation of fibrinogen by ferment and by heating to 56°–60° C., is the same, resulting in the formation of an insoluble product, relatively rich in nitrogen, and a soluble globulin, relatively poor in nitrogen, and coagulating at a higher temperature than fibrinogen. He does not believe that the fibrinogen molecule splits up directly, yielding those two products. If a definite decomposition of this sort occurred, the relative proportions of the two products formed would remain constant, whereas they may vary within wide limits. He thinks rather, as stated above, that from the larger portion of the fibrinogen molecule the fibrin or coagulated fibrinogen is formed by the action of the ferment or heat, while the portion of the fibrinogen molecule left undergoes a further change, resulting in the formation of the second soluble product.

The essential point of difference between the two theories lies

in the rôle attributed to paraglobulin. According to Schmidt, paraglobulin takes a direct part in the reaction, and in some way or other is represented in the fibrin molecule; while according to Hammarsten the paraglobulin takes no direct part at all in the process, the fibrin is formed from the fibrinogen alone under the influence of the ferment.

The chief arguments advanced by Schmidt in support of his view are these: (1) There are certain hydrocele liquids which contain either no paraglobulin at all, or, at the most, unimportant traces of it. If we add to such hydrocele liquids a solution of ferment alone, no coagulation takes place, while the addition of serum readily causes the formation of a clot. (2) The weight of fibrin formed increases to a certain extent with the quantity of paraglobulin added.

With regard to the first of these statements, Hammarsten has shown conclusively that it is erroneous. Hydrocele liquids which do not clot spontaneously, nor upon the addition of ferment solutions prepared according to Schmidt's method, are found upon analysis to contain much more paraglobulin than fibrinogen. The paraglobulin of such liquids, moreover, is not fibrinoplastic; that is, if a pure solution of it is prepared from the hydrocele liquid and added, together with some of Schmidt's ferment solution, to another hydrocele or pericardial liquid which does not clot spontaneously, nor upon the addition of ferment alone, no coagulation follows; while if some blood-serum is added to the same liquid a clot is obtained.

While these experiments completely disprove Schmidt's statement that the reason such hydrocele liquids do not clot with ferment alone is that they contain no paraglobulin, it still remains a question why they coagulate with blood-serum and not with the artificial serum made from the ferment and the hydrocele paraglobulin. According to Hammarsten, hydrocele liquids contain some substances which prevent the formation of fibrin, or, at least, prevent its separation in an insoluble form, and he gives a number of experiments to show that this is the case. The injurious action of these substances is prevented in some way by paraglobulin. When serum is added, therefore, the ferment present is allowed to work on the fibrinogen and fibrin is formed. The paraglobulin favors the formation of fibrin indirectly. The

reason that a ferment solution, plus non-fibrinoplastic paraglobulin, prepared from hydrocele liquids of the kind described, does not cause coagulation when added to other hydrocele liquids of the same character, appears to be that the solution is too weak in ferment to act upon the fibrinogen under these conditions. The paraglobulin solutions prepared from such sources probably contain little, if any ferment, and the ferment solution when made according to Schmidt's method is usually not very strong in ferment—not nearly so strong as blood-serum. In support of this view, Hammersten has been able, by a new method (3), to prepare a ferment solution which in successful cases is very powerful, and when added alone to hydrocele liquids of the kind described gives a clot.

With regard to Schmidt's second argument, Hammarsten admits that the addition of paraglobulin may increase the amount of fibrin which can be obtained from a given weight of fibrinogen. He explains this result upon the ground that the paraglobulin simply prevents the unfavorable action of substances present in the plasma or fibrinogenous liquid used. Amongst such substances he names the alkalies and alkaline salts, though there are others as yet unknown. He finds that the amount of fibrin may be increased by other means; for instance, simple neutralization of the liquid, or the addition of $CaCl_2$, or of an impure casein prepared by a special method. Hammarsten's experiments on this point are not so complete as might be desired, and this action of paraglobulin forms the strongest argument at present for those who believe that it takes a direct part in the formation of fibrin. In support of his theory of coagulation, however, Hammarsten brings forward the very strong positive proof that fibrinogen alone under the influence of ferment alone yields a clot containing typical fibrin.

His experiments were made entirely on mammals, and have never, so far as I know, been confirmed by any other investigator. I have, therefore, in connection with some work upon the blood of the terrapin, taken the opportunity of repeating his experiments. The results of my work confirm on the whole Hammarsten's theory, as far as the formation of fibrin from fibrinogen alone is concerned, though in some minor points I have obtained results differing from his.

The method used in preparing the fibrinogen solution was essentially that given by Hammarsten (4). A cannula was placed in the pulmonary artery, and the blood allowed to flow directly into a glass cylinder packed in ice. From 50 cc. to 125 cc. of blood were usually obtained from a single animal. The blood was allowed to stand for 24–48 hours, and at the end of that time the corpuscles had settled to such an extent that an amount of clear plasma equal to about two-thirds of the original bulk of blood could be drawn off with a pipette.

In one case after the blood had been allowed to stand for 48 hours, surrounded by ice, and the greater portion of the clear plasma had been removed, the cylinder containing the blood was left in the refrigerator without any ice, at a temperature of 15°–20° C. for about a week. At the end of that time the clear plasma above the corpuscles was found perfectly fluid, and when removed, a portion of it kept for several days in a warm room refused to clot, while another portion mixed with a little clear serum from a clot of colorless plasma clotted in a few minutes. The white corpuscles had evidently settled so completely that the plasma contained no ferment at all. The observation gives a very interesting proof that the ferment is formed from the breaking down of the white corpuscles, and that the fibrinogen exists pre-formed in the plasma.

The clear plasma was filtered, to get rid of the white corpuscles, in a funnel surrounded by ice. An equal bulk of saturated NaCl solution was then added, the solution quietly stirred and allowed to stand in the cold until a flocculent precipitate of fibrinogen had separated out. The fibrinogen was filtered off through a plaited filter, the filter carefully pressed between sheets of filtering paper, and finally cut into small bits which were stirred in a 6–8 per cent. solution of NaCl; the amount of NaCl solution used was about one-third that of the original plasma. After standing a few minutes the solution was filtered off and put through the same process a second and third time. The third precipitate after being pressed was dissolved in water, the fibrinogen going into solution readily by the aid of the small amount of NaCl still adhering to the filter. The solution finally obtained was colorless or very faintly opalescent, when good quantitative filter paper purified by HCl was used. The

solutions were absolutely free from paraglobulin, since they were completely precipitated by saturation with NaCl or by heating to 60° C.

For the preparation of ferment I have used several different methods, but obtained the best solutions from Schmidt's well known process of precipitating the proteids of serum by alcohol. Hammarsten's new method of preparing the ferment gave, for the few times that I tried it, very weak solutions. The method consists in throwing down the paraglobulin from serum by saturation with $MgSO_4$, precipitating the $MgSO_4$ and ferment by sodium hydroxide, dissolving the magnesium hydroxide in acetic acid, and dialysing off the magnesium acetate. The solution finally obtained is very strongly diluted, owing to the high osmotic equivalent of magnesium acetate; its weak ferment action is most probably caused by this fact.

The solutions of ferment prepared by Schmidt's method gave no opalescence whatever when heated to 80° C. When boiled, a very faint opalescence was sometimes seen, while in other specimens even boiling did not cause the slightest turbidity, although the solution was perfectly neutral, so that there could be no question of the absence of even traces of paraglobulin. When solutions of such ferment were mixed with the pure fibrinogen, a firm colorless clot was obtained in all cases; the time necessary for the formation of the clot varying from ten minutes to an hour, at the ordinary temperature of the room.

There can be no doubt then, that with the blood of this animal as with that of the mammal, fibrin is readily formed from pure fibrinogen under the influence of the ferment. The fibrin formed in this way, however, usually appeared more soluble in dilute NaCl solution than that formed normally from the blood, resembling in this respect the fibrine concrète pure of Denis obtained under certain conditions from the venous blood of man. Hammarsten noticed the same fact in some of the specimens of fibrin obtained from his fibrinogen. He states that a fibrin of this character may be formed under three conditions, viz., by the addition of large quantities of paraglobulin to the solution, by the addition of small quantities of free alkalies, or normally from certain specimens of fibrinogen which in this respect are not quite typical. The clots of fibrin obtained from my fibrinogen solutions by the

action of ferment quite often dissolved to a turbid liquid in the course of 48 hours, while the clot formed from the clear plasma was much more stable. Whether this difference was caused by the presence of the paraglobulin in the plasma or by the greater strength of the plasma in ferment and fibrinogen I am unable to say.

In one respect the fibrinogen solutions which I obtained from the terrapin's plasma differed markedly from those obtained by Hammarsten from mammalian plasma. As was said in speaking of his theory, the fibrinogen prepared by him was only partially coagulated by heating to 60° C., a small portion of the original fibrinogen remaining in solution as a new globulin, coagulating at 64°–66° C. The reactions of this globulin resemble very closely those of fibrinogen; this is especially seen in its behavior towards salt solutions. Hammarsten has not shown that a globulin of this character does not already exist in the plasma, and an objection to his experiments might be made on this ground. If such a globulin is present in the plasma, it will be precipitated, together with the fibrinogen, in the method used for preparing the latter—and will appear in the final solution. The fibrin formed from the solution under these conditions might be considered as the result of an interaction between the fibrinogen and this third globulin under the influence of the ferment. Or this body might possibly be regarded as a modified paraglobulin changed by the method of preparation. However that may be, the same objection cannot be raised against the fibrinogen solutions prepared from the terrapin's blood. This fibrinogen is completely precipitated from its solutions by heating to 56°–60° C. The filtrate from the precipitate when boiled gave in many cases no opalescence whatever, and no proteid reaction with the xanthoproteic test. Sometimes the filtrate gave when boiled a very faint opalescence, so extremely small, however, that it could be neglected. I have found also that fibrinogen prepared in the same way from hydrocele liquids which are not spontaneously coagulable, is likewise completely precipitated when heated to 60° C.

On the other hand, when a solution of fibrinogen was allowed to clot with ferment and the fibrin removed as quickly as it was formed to prevent any of it from being dissolved, it was found, in

accordance with Hammarsten's statement, that only a portion of the fibrinogen was converted into fibrin (see preceding paper, p. 58), the remainder staying in solution as a new globulin coagulating not at 64°–66° C. as is the case with mammalian fibrinogen, but at a temperature between 75° and 80° C. Hammarsten's statement that the coagulation of fibrinogen by heat and by ferment action is essentially the same process, will not hold with terrapin's blood at least, and to judge from the action of the hydrocele fibrinogen is not true for human blood either.

The third globulin formed from a portion of the fibrinogen when exposed to the action of ferment cannot be explained as a portion of dissolved fibrin, since I found that in one case, in which the fibrin formed could be dissolved in 2 per cent. NaCl solution, it was completely re-precipitated when heated to 64°–65° C.

Several series of experiments were made to determine at what temperature the action of the ferment is destroyed. The ferment solution used in these experiments was a second extract of the coagulated proteids of the serum obtained by Schmidt's method, and was not very strong. Small portions of the solution were exposed to different temperatures during five minutes, allowed to cool, and then mixed with a solution of fibrinogen. It was found that up to 70° C. the action of the ferment is not destroyed by heating for five minutes, although apparently weakened, since a longer time is required for coagulation, and the clot formed is more or less imperfect. When heated to 80° C. for five minutes its power is entirely lost. A short exposure of one or two minutes to even 100° C. does not completely destroy the ferment, a small clot can still be obtained with fibrinogen.

The aqueous solution of the ferment differs remarkably from blood-serum in its behavior toward heat. When portions of the serum are heated for five minutes to 50° C., and, after cooling, mixed with fibrinogen solutions, coagulation takes place only after 48–72 hours, and if heated to 60° C. for the same time no clot at all can be obtained with it. Hammarsten found that when mammalian serum is heated to 58°–60° C. it loses its fibrinoplastic power, *i. e.* the ferment is destroyed, while the properties of the paraglobulin remain unaltered. The fact that the ferment is destroyed more easily when dissolved in serum than in its aqueous solutions may possibly be explained by the alkalinity of the serum.

The ferment of terrapin's blood does not appear to differ in any respect from that of mammalian blood. Hydrocele liquids clot readily with terrapin's serum, and, on the other hand, solutions of fibrinogen prepared from terrapin's plasma, when mixed with ferment made from the blood of a dog or ox by Schmidt's method, coagulate without difficulty.

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ON THE ACTION OF CARBOLIC ACID, ATROPIA AND CONVALLARIA ON THE HEART; WITH SOME OBSERVATIONS ON THE INFLUENCE OF OXYGENATED AND NON-OXYGENATED BLOOD, AND OF BLOOD IN VARIOUS DEGREES OF DILUTION. By H. G. BEYER, M. D., M. R. C. S., Passed Assistant Surgeon, U. S. N.

The experiments described in the following pages are for the most part rather of therapeutical than of purely physiological interest. They were, however, carried out in the physiological laboratory of the Johns Hopkins University, whose facilities for such work were placed at my disposal by Professor Martin. I have, accordingly, desired to publish them in this Journal.

The method of work employed has already been very well described by Howell and Warfield (*Studies from Biol. Lab.*, Johns Hopkins University, Vol. II, p. 329), and also by H. H. Donaldson and L. T. Stevens (*Journal of Physiology*, Vol. IV, p. 165). A full description of it can, therefore, be here omitted. There are, however, one or two minor points in the operation for isolating the heart of the terrapin, and preparing the same for as nearly as possible normal and uniform work, to which it is desirable to call attention.

When the plastron of a terrapin is removed, one can easily watch its heart beat through the transparent pericardium, filled with clear lymph. Careful observation shows that during systole the base of the ventricle broadens out and, with its attached auricles and blood-vessels, moves slowly but steadily in a direction towards the apex. The apex, on the contrary, is fixed in position by a narrow strip of fibrous tissue, which arises from a tough membrane stretching across anterior to the liver and is inserted into the apex of the ventricle. Out of nearly sixty terrapins which I had occasion to examine, this band was missing in only one. By fixing it with ligatures in, as nearly as possible, the place it had occupied before the operation of tying in cannulas, &c., the relation of the entire heart to the vessels springing from it, especially the aortæ, seemed much more secure, and no tilting up of the apex to interfere with the outflow could occur. Furthermore, as far as I know, only one venous or in-

flow cannula, and one arterial or outflow cannula have hitherto been used by experimenters; I have used for the purpose of feeding the heart, cannulas in two or more of the veins passing to the heart, and for the outflow have inserted cannulas in two or all three of the arterial stems arising from the ventricle. More natural conditions are thus afforded to the isolated heart.

The Influence of Mammalian Blood in different Degrees of Dilution upon the Work done by the Heart of the Slider Terrapin.

Rossbach (*Arb. aus d. physiol. Institut zu Leipzig*, 1874, Bd. ix, p. 90) found that the isolated frog's heart under the influence of defibrinated blood beat regularly, but that under serum its beats occurred in groups. Klug (*Arch. für Anat. u. Phys.* 1879, p. 435) was unable to confirm these results, but found that whether blood or serum were used, pulsations either in groups or at regular intervals might be obtained.

Since the heart of cold-blooded animals was first successfully isolated and kept alive for a number of hours outside the body while fed by some nutritive liquid, as serum, blood, &c., a variety of feeding fluids have been tested, with the object of ascertaining which would cause the heart to yield the maximum amount of work in a given time. Thus McGuire (*Arch. f. Anat. u. Physiol.* 1878) obtained the maximum amount of work by feeding the heart with a mixture composed of one part of mammalian blood and two parts of a 0.6 per cent. sodium chloride solution; and C. S. Roy (*Jour. of Phys.* 1883) mentions that his experiments led him to the same conclusion. Kronecker, in reporting on some investigations of Mr. May's (*Arch. f. Anat. u. Physiol.* 1883, p. 263) states that the maximum amount of work done, and the greatest number of pulsations, were obtained when the heart was fed with undiluted blood. H. H. Donaldson and L. T. Stevens (*Jour. of Phys.* Vol. IV, p. 165) in their experiments on the influence of digitaline on the heart of the frog and terrapin, made use of a mixture of equal parts of blood and 0.75 per cent. salt solution. The heart was kept under observation in some of their experiments for over ten hours, and had in the meantime been repeatedly drugged, and still was reported as doing well at the end of that time. After having made a rather large number of experiments on this point in my preliminary work on

the frog and slider terrapin, substituting the saline mixture proposed by Sydney Ringer * (*Journal of Physiology*, Vol. III, p. 39) for the 0.75 per cent. of normal salt solution as used in the experiments of Donaldson and Stevens, I feel very safe in saying that so far as defibrinated calf's blood is concerned, the maximum amount of work and the greatest number of pulsations can be obtained from the heart by feeding with a mixture of about equal parts of blood and Ringer's solution. Any greater dilution results sooner or later in undue dilatation, first of the sinus, then of the auricles, and at last of the ventricle. A mixture of one part of blood and two parts of the saline may give for the period of an hour as much, or even a slightly larger, amount of work, but it meanwhile injures the muscular substance of the heart, the results being undue relaxation and irregularity in its action; on the other hand undiluted defibrinated calf's blood, in my hands at least, has not given either the maximum amount of work or the greatest absolute number of pulsations. In experiment I, besides undiluted defibrinated calf's blood, six different dilutions were employed, their strength varying as stated in the last column of the table, p. 77.

The results show that the maximum amount of work is not done by the heart when supplied with simple defibrinated calf's blood, and that under almost any of these dilutions more work is done in a given time than with the undiluted blood. When the mixture 1 : 20 was turned on, the heart continued its regular beat for 10 minutes, and then suddenly became irregular in its action, and unable to overcome the hydraulic pressure opposed to its contraction; it partially recovered, however, under a mixture of three parts of blood to one of Ringer's solution.

In all the experiments described in this paper the "venous pressure" indicates the height above the heart of the bottom of the air tubes of the supplying Marriotte's flasks. For example, "venous pressure 7 cm." means that the liquid supplied to the heart entered the cannulas supplying that organ under the pressure exerted by a column of the nutrient liquid seven centi-

* The composition of this mixture is:

Normal salt solution (0.75 per cent.), . . .	100. cc.
Calcium chloride sol. (1.390), ...	5. cc.
Sod. bicarbonate sol. (0.50 per cent.), . . .	2.5 cc.
Sol. of potass. chloride (1.0 per cent.), . . .	0.75 cc.

metres in height, and so on. When different liquids were used they were contained in separate Marriotte's flasks, carefully adjusted so that the pressure under which liquid flowed out of them was the same for all. The supply tubes from these flasks had stop-cocks on them, and all met in a common tube from which in turn the heart was supplied. By closing and opening the proper stop-cocks, any one flask could at will be used to feed the heart. All the Marriotte's flasks stood on the same horizontal platform, and were raised or lowered equally by moving this platform, if it became desirable to change the venous pressure in the course of an experiment. The outflow cannulas coming from the aortæ, or, in some cases, also from the pulmonary artery, were all connected with a single tube, from the distal end of which the liquid pumped round by the ventricle flowed out, and was collected and measured. The height of the orifice of this tube above the heart is stated in each experiment as the "arterial pressure." Being kept constant throughout an experiment, any variation in the weight of blood pumped out in a unit of time was proportional to the variation in the work done: that is to say, the "lift" remaining the same, any change in the "work" was indicated by, and was directly proportional to, variations in the "load" lifted. As the specific gravity of the various liquids used in any one experiment differed very slightly, the "load" lifted was practically proportioned to the bulk of the liquid pumped out by the ventricle. Accordingly it is stated in the third column of each of the tables of experiments, in cubic centimetres. The temperature stated in the fourth column is that of the liquid supplied to the heart. It never differed more than 0.5° C. from the temperature of the box in which the terrapin was enclosed, and gives more accurately the actual temperature of the heart. A mercury manometer was connected with the outflow tube near the heart. Its pen wrote on the smoked paper of a revolving cylinder, on which also a chronograph recorded seconds.

Experiment I.

March 6th, 1884. Terrapin, weighing 585 grms. Inflow cannulas in inferior vena cava and left superior vena cava; outflow cannulas in aorta and pulmonary artery. Nutrient liquid, calf's blood mixed with Ringer's saline in various proportions. Venous pressure, 7 cm. Arterial pressure, 25 cm.

Time. P. M.	Rate per min.	Work in C. C. per min.	Temp. Cent.	
3h. 00m.				Terrapin in box. Nutrient, undiluted defibrinated calf's blood.
30	31	31.5	21	
35	31	32		
40	31	32		Nutrient, blood and Ringer's solution (1 : 1).
43	32	45.5		
45	32	43.5		
46	32	44.5		Nutrient, blood 3, Ringer's solution 2, distilled water 1.
48	32	44.5		
51	32	44.2		Nutrient, undiluted blood.
56	31	31.5		
58	31	32		
4h. 00m.	31	31.5		
03	31	32		Nutrient, blood and Ringer's solution (1 : 1).
05	32	47.5		
07	32	45		
09	32	45.5		Nutrient, undiluted blood.
12	32	33		
16	32	33.5		
20	32	33.5		Nutrient, blood 3, Ringer's solution 2, distilled water 1.
23	33	47.5		
4h. 25m.	33	44	20.5	
27	34	42		Nutrient, blood and Ringer's solution (1 : 1).
30	34	42		
32	34	45		Nutrient, undiluted blood.
36	32.5	33.5		
39	33	34		
41	32.5	33.5		
43	33	34.5		Nutrient, blood and Ringer's solution (1 : 1).
46	34	47.5		
48	34	45		Nutrient, undiluted blood.
52	33	34		
55	33	34.5		
58	33	34		
5h. 00m.	33	34.5		Nutrient, blood 3, Ringer's solution 2, distilled water 1.
03	33	45.5		
05	35	42		Nutrient, undiluted blood.
09	33	33.5		
12	33	33		
14	33	33.5		
16	33	33.5	20.5	Nutrient, blood and Ringer's solution (1 : 1).
20	34	42.5		
21	35	43		Nutrient, undiluted blood.
25	32	34		
28	33	34		
31	33	34.5		Nutrient, blood 3, Ringer 2, distilled water 1.
33	34	47.5		
35	35	45.5		
37	35	44.5		Nutrient, undiluted blood.
40	33	34.5		
42	33	34.5		

Time. P. M.	Rate per min.	Work in C. C. per min.	Temp. Cent.	
5h. 44	33	35	20.5	
48	32	34		Nutrient, blood and Ringer's solution (1 : 1).
50	33	45		
53	34	44.5		Nutrient, undiluted blood.
57	33	34.5		
6h. 00m.	32	34		Nutrient, blood 3, Ringer 2; dist'd water 1.
02	33	45		
04	34	42.5		
06	34	43.5		Nutrient, undiluted blood.
09	32	34		Nutrient, blood and Ringer's solution (1 : 1).
14	34	45		Nutrient, undiluted blood.
19	32	34.5		
21	32	35		Nutrient, blood and Ringer's solution (1 : 1).
25	33	40.5		
27	34	40.5		
29	34	41		Nutrient, blood 3, Ringer 2, dist'd water 1.
33	34	45		Nutrient, blood and Ringer's solution (3 : 1).
36	34	42		
38	34	43		
41				Nutrient, blood and Ringer's solution (1 : 1).
44	33	42	20	
46	34	42		
49.30	34	42		Nutrient, blood and Ringer's solution (1 : 3).
52	35	49.5		
55	33	43		Nutrient, blood and Ringer's solution (3 : 1).
7h. 00m.	32	37		Nutrient, blood and Ringer's solution (1 : 3).
03	32	48		Nutrient, blood and Ringer's solution (3 : 1).
06	32	45	20	
07				Nutrient, blood and Ringer's solution (1 : 3).
23	32	50		
26	32	48		
29.30	31	46		
31	31	40		Nutrient, blood and Ringer's solution (3 : 1).
34	31	41		
38	32	48		
41	31	44		
46	31	45		Nutrient, blood and Ringer's solution (1 : 1).
50	31	39		
53	31	40		Nutrient, blood and Ringer's solution (1 : 7).
56	33	50		
8h. 00m.	32	45		
05	31	44		Nutrient, blood and Ringer's solution (1 : 20).
10	30	41		
12				Heart becoming distended and not contracting properly.
14	30	42		
18				Two ventricular to each auricular contraction.

An attempt was made to recover the heart by supplying it with a mixture of blood and Ringer's solution (3 : 1), but without success.

The preceding experiment seemed to show so clearly the su-

periority of defibrinated blood and Ringer's solution mixed in equal volumes, that I used this mixture in all subsequent experiments.

The Influence of Oxygenated and Non-oxygenated Blood upon the Work of the Heart.

One of the first to investigate the influence of different gaseous substances on the isolated heart of cold-blooded animals was Castell (*Arch. f. Anat. u. Physiol.* 1854, p. 226). According to this observer the heart of the frog, when cut out and placed under a bell glass, would on an average continue to beat for three hours, the ventricle ceasing its contractions long before the auricles. In rarefied air the heart beat only for thirty minutes. In an atmosphere of oxygen the heart was found to beat for twelve hours; surrounded by carbon dioxide gas, the contractions continued only six minutes; they were, however, resumed on the admission of air, and then continued for two hours. Cyon (*Comptes Rendus*, 1867), from some experiments made in the laboratory at Leipsic under Prof. Ludwig, came to the following conclusions: "Mes expériences ont demontré que l'oxygen excite surtout les ganglions moteurs du cœur tandisque l'acide carbonique agit de la même manière sur les ganglions regulateurs." McGuire (*Arch. f. Anat. und Phys.* 1878, p. 321), working under Kronecker's direction, arrived at the conclusion that oxygen exerted no perceptible influence on the heart's action, while carbon dioxide weakened it very much. A year later Klug (*Arch. f. Anat. u. Phys.* 1879, p. 435) published a number of experiments on the influence of oxygen and carbonic acid upon the frog's heart. The conclusions arrived at were, that oxygen very much increased both the intensity and the frequency of the heart's beat, while carbonic acid had the opposite effect. His experiments, therefore, were in perfect agreement with those of Castell made nearly twelve years previously. Some years later, Klug in connection with Desiter Velits undertook the same line of research on the mammalian heart. The gases were administered through the lungs, by means of an apparatus for artificial respiration which was in connection with gasometers containing oxygen and carbon dioxide gas respectively. It was found that in animals with divided spinal cord and vagi a 40 per cent. car-

bon dioxide mixture blown into the lungs produced no perceptible change for forty seconds. During the last third of the first minute and during the second minute the number of beats decreased, arterial pressure went down, and continued to fall until death ensued at the end of five or six minutes. In animals treated with oxygen, the pulse rate was considerably increased and arterial pressure raised. Klug and Velits concluded that oxygen exerted a stimulating influence upôn the intracardiac nerve-centres, while carbon dioxide paralyzed them. Relative to the influence of carbon dioxide upon the intracardiac nervous apparatus, I find the following statement in an article by Dastre and Morat, who exposed the heart to asphyxiated blood: "Il est possible d'admettre au moins dans certain cas une excitation direct des centres nerveux intracardiacques, mais cette effet est extrêmement tardif, souvent obscur, et il n'intervient en definitive que par une faible part dans le processus asphyxique du cœur." According to Dastre and Morat asphyxiated blood has a stimulant action upon all the tissues (*Archive de Phys. norm. et path.* 3m. ser., tome 3, 1884). Granting that there exists a certain amount of evidence in favor of each of the opinions quoted in this short review of the literature of this important subject, it is nevertheless certain that oxygen cannot stimulate and be indifferent to, or carbonic acid stimulate and paralyze the same tissues at the same time and under the same circumstances. When in addition Paul Bert states that oxygen under several atmospheres of pressure exerts a poisonous action on living tissues, the strong and just criticism his assertions met with from Cyon (*Arch. f. Anat. et Phys. Supp. Bd.* 1883), and from Lehmann (*Pflüg. Arch.*, Vol. XXXIII, p. 173, 1884), might have been foreseen. Cyon and Lehmann have definitely established the fact that oxygen, even under very high pressure, does not destroy the vitality of a tissue, but may render its manifestation latent. Lehmann after exposing hearts at a somewhat low temperature to a pressure of thirteen atmospheres of oxygen, still obtained a reappearance of regular pulsations after the lapse of twelve hours, and lasting for days.

The experiments which I am about to describe show the very decided influence which is exerted on the heart's action by even very small doses of oxygen and carbonic acid respectively. The

way in which asphyxiated and oxygenated blood were prepared is as follows: a quantity of defibrinated calf's blood previously mixed with an equal volume of Ringer's saline was divided into two portions, and each portion put into a bottle; one was allowed to stand quiet while the other was shaken up for a few seconds every now and then, until a perceptible difference in color was noticed; this was brought about in from five to fifteen minutes. Then the two bottles were turned into Marriotte's flasks by inserting the proper stoppers and tubes, and when the time came for an observation, the blood was allowed to run through the heart and its effect noted. From Experiment II it will be seen at a glance that the rate and the amount of work are always increased on supplying the heart with oxygenated blood. The rate, at first, very little, later on a difference of five beats per minute exists. The work done increases from 30 to 175 per cent. per minute. A very slight agitation of the blood before returning it into the flask will increase the work done by the heart, even when the difference in color is not perceptible to the eye. The importance of this point will be readily seen by those who have worked on the action of drugs on the heart. The plan of getting the drug into the blood followed out in all the experiments described later in this paper, was to dissolve it in Ringer's saline first, and then mixing it with defibrinated blood, required no more shaking than preparation of the control mixture of equal parts of blood and Ringer's solution; thus uniformity was reached in this respect. If the drug be directly added to the blood mixture and well shaken up with it, effects really due to better oxygenated blood may be ascribed to the drug under examination.

The following table gives the data of one experiment; several others were made which agree with it in all essential points.

Experiment II.

January 4th, 1884. Terrapin, weight 1100 grms. Calf's blood and Ringer's saline 1:1. Inflow cannulas in inferior vena cava and hepatic vein; outflow cannulas in left aorta and pulmonary artery. Venous pressure at start, 5 cm. Arterial pressure, 18.5 cm.

Time. P. M.	Rate per min.	Work in C. C. per min.	Temp. Cent.	The phrases "On CO_2 blood" and "On O-blood" in this column indicate that non-aërated and aërated blood respectively were supplied to the Heart immediately after the time stated on the same line in column one.
2h. 20m.				Terrapin in box.
3h. 20m.	22	49	20.5	
25	21	49		
30	21	49		On O-blood.
35	21	51		
40	21	53		Venous pressure lowered 1.5 cm.
45	21	49		
50	20	48		
55	21	48		On CO_2 blood.
4h. 00m.	21	38		
05	21	36		
10	21	37		Off CO_2 blood ; on O-blood.
15	21	46		
20	21	48		
25	21	48		
30	21	47		
40	21	40		On CO_2 blood.
45	21	26		
50	21	28		
55	21	24		
5h. 00m.	21	26		On O-blood.
05	22	44		
10	23	45		
15	24	47		On CO_2 blood.
20	22	15		
25	22	16		On O-blood.
30	25	45		
35	26	47	21	
40	27	48		On CO_2 blood.
45	23	17		
50	22	14		On O-blood.
55	29	46		
6h. 00m.	29	45		On CO_2 blood.
05	25	20	21	
10	25	23		On O-blood.
15	29	47		
20	29	45		On CO_2 blood.
25	26	19		
30	25	19		On O-Blood.
35	29	45		

The experiment was continued until 8.30 P. M. with like results.

The Influence of Carbolic Acid upon the Heart of the Terrapin.

Notwithstanding the great medical and surgical importance to which carbolic acid has risen within the last two decades, and in spite of the deaths caused by it, which from time to time have been recorded, its physiological effects upon the circulatory

apparatus, more especially on the heart itself, seem as yet not very satisfactorily determined. Opinions are still at variance as to whether carbolic acid introduced into the animal organism may or may not be at first a stimulant to the heart and blood-vessels, thus increasing the blood pressure; or whether its depressing and poisonous effects are exhibited at once. Hoppe-Seyler (*Pflüger's Archiv*, v. 1872) obtained a rise in arterial and venous pressure just before convulsions came on; Salkowsky (*Pflüger's Archiv*, v. 1872, p. 344) noticed an increase in the rapidity of the blood-flow in the capillaries of the frog's web, followed by a slowing of the flow. Labbée (*Archives générales*, 6m ser., tome 18, p. 451, 1871) also noticed an increase in the strength of the systoles in the early stages of the poisoning and a contraction of the vessels. On the other hand, Rudinger (*Einige Beiträge zur Lehre d. Wirkung d. Carbolsäure*, Greifswald, 1874) noticed a slowing in the circulation in the web of frogs under the influence of carbolic acid, and Gies (*Zur Kenntniss d. Wirkung d. Carbolsäure, auf d. Thier Organismus, Archiv. f. exp. Path. u. Therapie*, xii, S. 401) found that lowering of the blood pressure and the decrease in the pulse rate took place almost simultaneously with the introduction of carbolic acid into the circulation, and that after a time the normal pressure and pulse-rate returned. If we add to this the observations of Salkowsky, Labbée, and a few others, that circulation survives respiration in carbolic acid poisoning, we have in few words about all that is known about the effects of carbolic acid on the circulatory apparatus. So far as the heart itself is concerned the subjoined experiments III and IV (which are selected from a considerable number, all concordant) show, first, that carbolic acid in the smallest, as well as in the largest doses, acts as a depressant from first to last, reducing its rate of beat and its work. Second, that the heart as long as it is supplied with well oxygenated blood, will, up to a certain degree, show what almost amounts to an immunity from the poisonous effect of the drug.

Experiment III.

January 18th, 1884. Terrapin, weight 935 grms. Calf's blood and Ringer's saline 1 : 1. Inflow cannulas in inferior vena cava, left superior vena cava, and hepatic vein; outflow cannulas

in right and left aortæ and pulmonary artery. Venous pressure, 4 cm. Arterial pressure, 24 cm. Carbolized blood contained 0.1 per cent. of carbolic acid in the first part of the experiment, and 0.2 per cent. in the second.

Time. P. M.	Rate per min.	Work in C. C. per min.	Temp. Cent.	
3h. 00m.				Terrapin in box.
45	16	34.5	20.5	Oxygenated blood mixture turned on.
4h. 00m.	18	54.5		Same blood returned without shaking.
15	17	45.5		
20	16	41.5		
22				On oxyg. blood mixture.
25	19	49.5		
30	18	50.2		On non-oxyg. blood.
35	18	44.5		On oxyg. blood.
40	19	52.5		On non-oxyg. blood.
45	18	46.5		On oxyg. blood.
50	20	54.5		
55	20	56.8		On non-oxyg. blood.
5h. 00m.	20	50		On carbolized oxyg. blood (0.1 : 100 cc.)
05	20	37.5		On oxygenated blood.
10	19	52.5		On carbolized non-oxyg. blood.
15	18	39.5		On oxygenated blood.
20	19	52.5		
25	20	55.5		
30	21	58.5	21	On carbol. non-oxyg. blood (0.2 : 100 cc.)
35	10	15.5		On oxygenated blood.
40	18	54.5		
45	20	57.5		
50	21	57.5		
55	21	57.		On carbol. blood (0.2 : 100 cc.) oxyg.
6h. 00m.	15	25.5		
02	15	23.		On oxyg. blood.
06	19	58.5		
10	21	60.		On carb. oxyg. blood (0.2 : 100 cc.)
15	18	30.5		
16.30				On oxyg. blood.
20	20	57.8		
25	21	59.2		Ended experiment.

Experiment IV.

January 16th, 1884. Terrapin, 1235 grms. Calf's blood and Ringer's saline. Cannulas in left superior vena cava, hepatic vein, inferior vena cava, right and left aortæ and pulmonary artery. Carbolized blood used in different strengths. Venous pressure, 6.5 cm. Arterial pressure, 22 cm.

Time. P. M.	Rate per min.	Work in C. C. per min.	Temp. Cent.	
4h. 20m.	16	32.5		Terrapin in box at 2h. 15m. Heart fed with oxygenated non-carbolized blood until 5h. 00m.
25	16	34		
35	16	34.5		
45	16	34	19.5	
50	16	33.5		
5h. 00m.	16	32.5		On carb. blood containing 0.1 : 500 cc.
05	16	18.5		On oxyg. blood.
10	16	32.5		
15	17	36		On carb. blood well oxyg. containing 0.2 : 500 cc.
20	19	45		On oxyg. blood.
25	18	38		On carb. blood, same as at 5h. 15m.
30	20	44.5		
35	20	46.1		On oxyg. blood.
40	21	38		
45	21	38		On carb. blood well oxyg., contain'g 0.4 : 500 cc.
50	21	43		
55	22	45		
6h. 00m.	22	48		
05	21	50		On oxyg. blood.
10	20	40		On carb. blood well. oxyg., contain'g 0.6 : 500 cc.
15	21	53		Heart slightly disturbed.
20	22	45		
25	21	43		
30	21	45	19.5	On blood well oxyg.
35	19	45		Heart contracting more perfectly.
40	20	42		
45	20	42		On carb. blood well oxyg., contain'g 0.8 : 500 cc.
50	12	23		On oxyg. blood.
55	13	23.5		Heart somewhat distended and contracting imperfectly ; on well oxyg. blood.
7h. 00m.	19	49.5		
05	19	48		On carb. blood same as at 6h. 45m.
10	10	22.5		
15	10	23		On well oxyg. blood.
20	18	46.5		
25	19	49		On carb. well oxyg. blood, contain'g 1.0 : 500 cc.
30	9	18.5		
35	11	21.5		
40	9	18.5		On well oxyg. blood.
45	17	45.5		Heart working very well.
50	17	45	20	On carb. blood same as at 7h. 25m.
55	9	19.5		On oxyg. blood.
8h. 00m.	15	42		
05	18	45.5		On carb. blood well oxyg., contain'g 1.2 : 500 cc.
10	7	18.5		Heart distended ; two faint auricular beats to one ventricular.
15	8	16.2		
20	5	10.5		On well oxygenated blood ; heart very much distended ; auricles at a stand-still ; ventricle beats once in two minutes. No blood pumped up to outflow orifice.
35	15	47	20	Heart has completely recovered.
40	16	45		
54	17	45		Experiment ended.

The two preceding experiments (and others which quite accord with them) seem conclusive that the depressant and paralyzing action of carbolic acid upon the heart can be considerably diminished, or be held at bay entirely, by a good supply of well-oxygenated blood; thus showing the great importance of the respiratory activity in cases of carbolic acid poisoning where there is danger from failure of the circulation and heart's action.

On the Action of Atropia on the Isolated Heart of the Terrapin and the Antagonism existing between it and Carbolic Acid.

Bartholow, in the fifth edition of his *Mat. Med. and Therapeutics*, makes the following statement: "I am indebted to Dr. A. C. Post, of New York, in a verbal communication, for the important fact that atropine is a physiological antagonist to the systemic symptoms induced by carbolic acid. He was induced to administer atropine in a case of poisoning by carbolic acid on observing the minutely contracted pupil and the failing circulation. The result was successful. Similar success has attended the same practice in other cases. Experiments on animals have also demonstrated the existence of this antagonism, which may now be regarded as an established fact." This statement, coming from and being endorsed by good authority, I determined to endeavor to find out in how far it might be true of the isolated heart. As far as experiments on animals are concerned, I am unable to find any published cases. In going over the literature on the physiological effects of atropia, I wish here, as elsewhere, to confine myself to that part of it which has reference to the circulatory apparatus. H. C. Wood (*Am. Jour. of Med. Sciences*, April, 1873), in four experiments on dogs whose pneumogastrics had been cut, obtained in one a slight rise, in the rest a more decided rise in arterial pressure and an increase in the frequency of the pulse rate; he believes that the enormous increase in the number of heart beats per minute seen in atropia poisoning is not entirely due, as has been believed, to paralysis of the vagi, but also to a direct stimulant action upon the antagonists of the latter. Von Bezold and Blœbaum (*Ueber die Wirkung d. Schwefel. Atropins*, *Unters. aus d. Phys. Lab. zu Würzburg*, *Heft* I), states that after section of the vagi and spinal cord, artificial respiration being maintained, atropia fails to increase arte-

rial pressure. Lemaitre (*Archives générales*, Aug. 1865, p. 49) claims to have shown that, notwithstanding division of the vagi, the action of the heart is still increased under atropia. Dr. John Harley, after experimenting on man, horse and dog, comes to the conclusion that atropia exerts a powerfully stimulating action on the heart (*Med. Times and Gazette*, March, 1868). Bartholow (Prize Essay, *Proceedings of the Am. Med. Association*, 1869) noticed an increased flow through the blood-vessels in frogs, with a slight contraction of the calibre of the vessels, which contraction was followed by an evident relaxation after some hours. In this Lemaitre, Minnot and Bartholow agree. In one of his experiments on frogs, Bartholow noticed, after the injection of $\frac{1}{4}$ grain of atropia sulphate, an increase in the pulse rate of from 40 to 52 per minute, also an increase in the contracting power of the heart. Schiff (*La Nazione*, 1872, No. 235) made the remarkable observation that a quantity of atropia slightly larger than that which is sufficient to dilate the pupil, lessens the sensibility of the heart to such an extent that the arterial pressure may be at first increased to three times its normal extent and then diminished to one-half or even one-third of that amount, without any change in the pulse rate being produced. If we assume that atropia paralyzes the extrinsic cardiac nerves, this is in accord with the experiments of H. Newell Martin on the isolated heart of the dog. Martin has shown (*Stud. Biol. Lab., Johns Hopkins University*, Vol. II, p. 213) that a heart set free from extrinsic nervous control is not affected as regards its pulse rate by variations in arterial pressure. Wood, as stated by Dr. Lauder Brunton (*London Med. Record*, 1873), found that in a certain class of frogs small doses (0.0001–0.001 gram) caused slowness of pulsation and sometimes complete stoppage in diastole, even after division of the vagi. After a shorter or longer stage the pulsations could be arrested by very weak interrupted currents applied to the venous sinus, but later, when the pulsations began to become quicker, stronger irritation was necessary to produce this effect. At the same time that the atropia produces slowness of the heart's beats and longer diastole, it makes the systoles stronger and longer. The phenomena have been interpreted as showing that atropia strongly stimulates the inhibitory and musculo-motor nerve tissues in the heart, the former predominating

over the latter. In another set of frogs, atropia seemed to paralyze these nervous tissues without previously stimulating them. In a third class the inhibitory centres were rapidly paralyzed while the musculo-motor ones were only slightly or not at all affected. In this class the end of the vagus in or near the heart seemed paralyzed before the inhibitory centre, so that, shortly after the administration of the poison, irritation of the nerve may have no effect, while the beat can still be arrested by irritation of the venous sinus. Von Bezold and Blœbaum found as the result of the smallest doses of atropia, in rabbits as well as in dogs, an increase in the frequency of the pulse rate and in the arterial pressure. In somewhat larger doses, an increase in the frequency of the pulse rate, but a fall instead of a rise in arterial pressure; still larger doses produced a decrease in the pulse rate, which, after some minutes, was followed by an acceleration and a fall in the pressure; the latter, however, rose again, but finally remained lower than normal. Finally, a dose of 0.1 gram, brought at once into the heart of the rabbit, paralyzed it immediately. They agree with Rossbach and Fröhlich in believing that atropia paralyzes the terminal filaments of the vagus within the heart. Meuriot, *De la methode phys. en Therapeutique et de ses applications à l'etude de la Belladonne*, Paris, 1868), formulates his conclusions as follows: small doses of atropia accelerate the heart and augment arterial pressure; the former is brought about by a paralysis of the terminal fibres of the pneumogastrics, the latter is due to increased muscular tonicity in the blood-vessels. In large doses, the muscular tonicity is impaired, and arterial pressure falls; the pulse rate, also, is diminished in frequency.

Experiment V.

March 4th, 1884. Terrapin; 845 grms. Calf's blood and Ringer's saline in equal parts. Inflow cannulas in left superior vena cava and inferior vena cava; outflow cannulas in left aorta and pulmonary artery. Venous pressure at first, 3.5 cm. Arterial pressure, 24.5 cm. The atropinized blood contained 0.002 grm. of atropia sulph. to 100 cc. of the blood mixture.

Time. P. M.	Rate per min.	Work in C. C. per min.	Temp. Cent.	The liquid stated in this column was supplied to the heart in each case immediately after the time stated on the same line in column one.
2h. 40m.				Terrapin in box, and heart fed with non-poisoned blood.
4h. 00m.	27	29	18.5	
05	27	29		
10	27	29.5		
15	27	30		
20	27	30		
25	27	31		Atrop. blood turned on.
28	27	31.5		On unpoisoned blood.
31	27	30		
34				On atrop. blood.
37	27	30		On unpoisoned blood.
42	27	31		
45	29	29.5		On atrop. blood.
50	27	31.5		On unpoisoned blood.
56	27	27.5		
5h. 00m.	27	27.5		On atrop.blood containing 0.004 : 100 cc. of blood.
02				On unpoisoned blood.
04	27	54		Ventricle somewhat distended.
08	27	38		
10	28	31	18	
20	28	25.5		On atrop. blood.
23				On unpoisoned blood.
28	28	28.5		
30				Raised venous pressure to 6 cm.
33	28	45		
38	28	44		
46				On atrop. blood.
48				On unpoisoned blood.
52	27	43	17.5	On atrop. blood.
54				On unpoisoned blood.
55				Pressure vacillating between 22 and 25 mm. Hg.
6h. 00m.	29	29		
02	28	22.5		
04				On atrop. blood.
06	28	44		
08				On unpoisoned blood.
15	28	38		
18	28	40		
23	27	35		
23.30				On atrop. blood.
25	28	40	17.5	
27.30				On unpoisoned blood.
36	27	48		On atrop. blood.
39	27	35		
41	27	40	17	On unpoisoned blood.
44	27	62.5		
52	27	42		
7h. 04m.	27	40		On atrop. blood.
06	27	41		
09				On normal blood.
23	26	35.5	16.5	
24				On atrop. blood.
28	26	40		
31	26	40		
32				On unpoisoned blood.
35	25	35		
44	26	31	16.5	Heart still working tolerably well. Ended experiment.

The preceding experiment includes twelve observations on the action of atropized blood on the heart. A small increase in the work done by the heart, due to the action of atropia, can, I think, be plainly noticed, more strongly marked with the stronger dose of the drug. Twice, however, a marked increase in the diastolic expansion of the ventricle occurred after turning on normal blood, and a consequent increase in the work done. This is, I think, to be explained in this way: atropia, being a stimulant to the heart muscle, causes the ventricle to work within certain limits of dilatation only; it checks the full diastole. The drug being suddenly withdrawn, the ventricle relaxes and receives more blood, without, however, being rendered unable to pump it out in its systole. At 5h. 04m., two minutes after normal blood was turned on, 54 cc. were thrown over in a minute, this quantity goes down steadily until at 5h. 20m. it has decreased to 25.5 cc. which was less than had been done at any previous time in the course of the experiment. At 5h. 30m. the venous pressure was raised, and consequently the quantity of blood received and pumped out by the heart increased. The heart then was atropinized without any marked increase in its work being observable. Unpoisoned blood mixture was then turned on, and this was followed by a considerable decrease in the work done until, at 6h. 02m., only half the original quantity of blood was pumped over in each minute. Atropinized blood being again turned on, the heart's work was brought up promptly to its former standard. This experiment shows well the stimulant action of atropia on the heart. When the heart is doing its normal work only a slight increase is observed under atropia. When, however, the muscular tone of the heart is lowered, then atropia exerts a powerful stimulating influence on the heart's action. The decrease in work, noted twice during the experiment after atropia was withdrawn, is probably due to the reaction which is apt to follow every stimulatory action on the tissues.

Experiment VI.

March 28, 1884. Terrapin, weight 1160 grms. Calf's blood and Ringer's saline (1:1). Inflow cannulas in hepatic vein, and inferior vena cava; outflow cannulas in left aorta and pul-

monary artery. Venous pressure, 7 cm. Arterial pressure, 30 cm. Carbolized blood contained 0.6 per cent. of carbolic acid. Atropized blood contained 0.02 grm. of atropia sulphate in 300 cc. of the blood mixture.

Time. P. M.	Rate per min.	Work in C. C. per min.	Temp. Cent.	The circulating liquids mentioned in this column were supplied to the heart immediately after the time stated on the same line in the first column.
4h. 00m.				Terrapin in box. Heart supplied with the normal blood mixture.
5h. 00m.	29	30	21	
10	29	31		
15	28	31.5		
25	29	31		
35	29	31		
45	28	31.5		
50	28	29.5		
57	28	30		On carb. blood.
				Carb. blood turned off at 5h.57′30″; on good blood.
58				Heart cavities over-distended and almost motionless.
6h. 00m.				Blood ceased to come over through outflow tube.
16				Blood begins to come, drop by drop.
20	30	35		
25	30	35		On atropized blood.
27	34	35		
29	34	36		
31				On normal blood mixture.
35	32	32.5	21	
39	32	37		On atropized blood.
42	33	37.5		
44	33	38		On normal blood mixture.
49	35	39		On atropized blood.
54	32	37.5		On normal blood mixture.
7h. 02m.	32	40		
06	33	40		On atropized blood.
10	34	37		
12	32	33		Heart smaller than before; contracting more vigorously.
15	32	33		On carb. blood.
15½				Carbol. blood off and atropized blood turned on.
16				No blood pumped out of outflow tube. Heart enormously distended, contractions of auricles scarcely perceptible, ventricular contraction peristaltic.
25				No blood coming over yet.
29				Blood begins to drop over.
36	28	25		Auricles still abnormally distended, but growing smaller after each contraction.
40	33	30		
43	34	29		On normal blood mixture.
47	34	17	20	Auricles twice as large as under atropia, ventricles smaller.
50	33	15		Auricles almost motionless.
51				On atropized blood.

EXPERIMENT VI.—Continued.

Time. P. M.	Rate per min.	Work in C. C. per min.	Temp. Cent.	The circulating liquids mentioned in this column were supplied to the heart immediately after the time stated on the same line in the first column.
				Auricles contract vigorously and completely at 7h. 52′ 30″.
7h. 55m.	34	31		On normal blood.
8h. 10	33	34.5		On atropized blood.
17	34	30		On normal blood mixture.
19				Auricles again abnormally distended.
21	34	30		Auricles still very large.
24	34	31		On atropized blood.
30	34	26		Auricles smaller on normal blood mixture.
34				Irregular ventricular contractions.
43	22	19		Auricles again enormously distended, irregular action of the heart, peristaltic waves passing in different directions.
9h. 06m.				Condition same. Experiment ended.

The preceding experiment shows, first, that after the heart has been carbolized, atropia increases its rate and also its work; second, that a more rapid recovery takes place after carbolization when blood containing atropia sulphate is supplied than under normal blood mixture. The experiment is one of several which illustrate the same facts.

Convallaria Majalis.

In 1858 two alkaloids were found in this plant, and their discovery was announced by Walz (*Deutsche Naturforsch. Versamml. Berichte*, 34, pp. 175–9). One was called convallarin, the other convallamarin. The former was said to act as a purgative, the latter to possess very decided action on the heart. The literature on the physiological effects of convallaria, as well as that of clinical observations, is of more recent date. The drug has been tested especially in Russia, whence it travelled westward. The principal Russian experimenters in this line are Troitsky Bogoiarlensky, Ysaieff, and Kalmykoff. Their results are mostly published in Russian, but from an extract in the *Revue des Sciences med. en France et a l'Étranger*, I find that a number of experiments on dogs and birds, as well as clinical observations on man, give the following results: Convallaria does not excite the cardiac ends of the vagus nerve, nor the cardiac accelerator nerves; it stimulates the central inhibitory

apparatus and paralyzes the intra-cardiac nerve-centres. The results obtained in Russia all agree in this respect. As regards arterial pressure, it rises in the beginning and falls later on; if the dose has been large the fall occurs at once.

The effects on other organs and systems do not immediately concern us in this connection. Stiller (*Versuche ueber Conv. maj. bei Herzkrankheiten*, *Wiener med. Wochenschrift*, No. 44, 1882) publishes the results of twenty-one observations, which are in direct contradiction with the clinical results obtained in Russia. Stiller says: In 17 cases the effect produced was absolutely nil, there was neither modification in the rhythm of the heart, nor a diminution in the number of pulsations, nor an increase in their strength. In 9 out of these 17 cases digitalis was afterwards used with marked success. Twice convallaria produced diuresis without any other effect. In France one of the principal advocates of convallaria is Prof. Sée. His experiments with it have been both physiological and clinical. He states that in the dog, it first slows the action of the heart and increases the blood pressure, respiration at the same time becoming slower and fuller. Toxic doses cause rapid and irregular pulsations, blood pressure still remaining above normal; finally arterial pressure falls, the pulsations grow feeble, and death takes place through syncope; the ventricle is arrested in systole, the auricles in diastole; the heart of the tortoise was found to possess more resistance to its action than that of other cold-blooded animals. He recommends convallaria, as indicated in all cases of cardiac disease and as counter-indicated in none. He also states that convallaria does not possess a cumulative action, but is very promptly eliminated from the system. Fillond Lavergne (*Étude sur le conval. maj.*, etc. Paris, 1883, *Thesis*) from some experiments and clinical results comes essentially to the same conclusions as Sée. Noguès (*Essai sur le convall. majalis*, Paris, 1883) arrives at the conclusions that the good effects of convallaria on patients affected with heart disease are inconstant, and may be depended upon only in some lesions very far advanced. Durieux disfavors convallaria (*Étude comp. du muguet et de la digitale*, *These de Bordeaux*, 1882), concluding from some physiological and clinical studies that under its influence an irregular and intermittent heart rarely recovers its normal

rhythm; pulse and temperature are not modified; the diuretic effect is inconstant. In every case of dropsy and œdema due to cardiac disease he states that the results have been unsatisfactory. This author very much prefers digitalis to convallaria. Desplats (*Journal des Sciences Med.*, Lille, 1882, *Action du muguet sur le cœur et sur les reins*), on the contrary, has found that convallaria has a decided effect upon the heart and kidneys, and he obtained in cases of cardiac disease in which it was administered, a slowing in the pulse rate, an increase in the energy and regularity of the heart, and an abundant diuresis. He adds, that if its use is continued more than eight or ten days without suspension, it produces a diminution in the force and energy of the heart. Marmé (*Schmidt's Jahrbücher*, Bd. CXXXIV. p. 166) found that convallaria kills by direct action upon the heart, and in moderate doses first slows and then quickens the pulse; previous division of the vagi does not interfere with these phenomena. In America, convallaria has likewise met with varying success, its virtues having been expounded by Taylor of New York, and questioned by Robinson of Philadelphia. With regard to its physiological properties, I. Ott (*Archives of Med.*, February, 1883) concludes that it increases arterial tension greatly at that stage of its action in which the heart begins to beat more frequently; that the subsequent decrease in cardiac frequency is not due to cardio-inhibitory excitation, but to an action on the heart itself, probably its muscular structure; that the rise in arterial tension is mainly due to stimulation of other vaso-motor apparatus than the main vaso-motor centre in the medulla oblongata.

Experiment VI.

February 15, 1884. Terrapin, weight 715 grms. Calf's blood and Ringer's saline in equal proportions. Inflow cannulas in inferior vena cava and left superior vena cava. Outflow cannulas in right aorta and pulmonary artery. Venous pressure, 4 cm. of the circulating liquid. Arterial pressure, 27 cm. Conv. blood contains 0.002 of convallamarin to 100 cc. of blood.

Time. P. M.	Rate per min.	Work in C. C. per min.	Temp. Cent.	
2h. 30m.				Terrapin in box. Heart supplied with normal blood mixture.
3h. 30m.	25	25	19.5	
35	25	24		
40	24.5	23.5		
45	24.5	23.5		
50	24	23.5		
55	24	23		
4h. 00m.	24	22.5		On conv. blood.
03	26	26		On normal blood mixture.
06	26	25.5		
08	26	26		
10	26	25.5		
12	25	25.5		
14	25	25.5		
16	25	25		
18	25	25.5	20	
20	25	25.5		
25	25	25.5		
26				On conv. blood, and kept on for 3 minutes; then substituted by normal blood mixture.
30	25	23.5		
35	26	23	20	
40	26	25		
45	26	25.5		
50	26	26		
55	26	27		
5h. 00m.	26	27.5		On conv. blood; kept on 6 minutes; then normal blood mixture turned on instead.
09	27	22.5		
10	27	24.5		
14	27	26.5		
20	27	28.5	20	
25	27	28		
28	28	27.5		
29	29			On conv. blood.
35	27	21		Auricles very small and contracting sluggishly.
39	28	19		Left aur. pale; right still contracting pretty well.
41	29	18		Ventricle $\frac{1}{3}$ its normal size, never fully expanding, but actively contracting.
43	29	18		
44				On normal blood mixture.
50	31	17		
53	34	18.5		
55	34	18		
58	34	18.5		
6h. 00m.	33.5	19		
03	34	20	20	
09	33	21.5	20	
13	33	22.5		Left auricle begins to expand and look normal.
19	33	25.5		
23	33	25.5		
28	33	27		Heart working with great vigor.
33	33	26.5		

EXPERIMENT VI.—Continued.

Time. P. M.	Rate per min.	Work in C. C. per min.	Temp. Cent.	
6h. 35m.	32.5	26.5		
40	32.5	26	20	
45	33	26		
46				On conv. blood.
50	33	21.5		
52	33	18.5		
55	35	16.5		On normal blood mixture. Entire heart much smaller than usual; very pale and bloodless; peculiar peristaltic but ineffectual contractions; both auricles shrunken.
57	36.5	0	20	
7h. 02m.	38	1.5		
05				Heart ceases to pump blood up to the outflow orifice; it contains some blood which it cannot propel; movements peristaltic.
12				No blood comes over yet. Raised venous pressure 2.5 cm.
14				Auricles slightly larger, but not working; ventricle again actively contracting.
17	33	2		Lowered venous pressure 2.5 cm.
20	33	2		Ventricle making 7 to 8 contr. to one auricular.
30	25	7.5		Left auricle and left side of ventricle bright red; rest dark red and apparently shrunken and not working.
45	33	9		
53	35	7		Left auricle and left side of ventricle working; right auricle and right portion of ventricle inactive and apparently beyond recovery.
8h. 00m.	34	6		
05	36	7		
10	38	7.5		Ended experiment.

All the rest of the experiments made with convallamarin, seven in number, show when small doses are used, a primary slight increase in the pulse rate and a slight increase in the work done; and a final arrest of both auricles and the ventricle in systole. Medium doses, or small doses often repeated, at first increase the rate, but diminish the work, and then arrest the ventricle in systole; the auricles, although much shrunken, being not so contracted as under a small dose. Large doses paralyze the heart in a short time, and arrest the heart at any point between systole and diastole.

In order to more accurately determine the influence of convallamarin on the contractions of the heart of the terrapin, the apparatus invented by Roy (*Journal of Physiology*, Vol. I, p. 452) was used in a somewhat modified way. The heart of the terrapin

being considerably larger than that of the frog, a wide-mouthed glass bottle was taken and its bottom cut off, a rubber cork was then tightly fitted in and perforated for the reception of two glass cannulas, one of which was introduced into the sinus, the other into one of the aortae; the heart was now filled with blood from a Mariotte's flask under a pressure of six cm. and immersed in the glass bottle which had previously been cemented on the brass stand and filled with blood; the base line was determined from the lowest extremity of the tracings on the drum, taken under an outflow pressure of about fifteen cm., which was afterwards lowered to ten cm. After having run through normal blood for fifteen to twenty minutes and finding no leak and no change in the tracings, convallamarin blood was substituted. Out of ten experiments made in this way three only were successful, on account of leakage. The tracings of one of the successful experiments hereto appended (Pl. VII, Fig. 3) show in a typical manner what occurred in all the three; namely, a gradual diminution of the volume of the heart and a steady decrease in the extent of its rhythmic variations in volume, until finally the entire heart, including sinus, auricles and ventricle, became very much shrunken and was arrested in systole. In one case one of the auricles was distended and showed no contraction; on further examination it was found filled with dark blood which could not escape on account of a slight twist which had occurred, preventing the free passage of blood from auricle to ventricle.

My experiments with convallamarin, so far as the isolated heart of the terrapin is concerned, seem to indicate that it increases the pulse rate, at least for a time, and slightly increases the work done; but that auricles and ventricle are arrested in systole when medium doses, or repeated small ones, are used. Large doses arrest the heart at once. The drug probably produces these results by a direct action on muscular substance of the heart. It has a decided cumulative action, acting more slowly than digitalis, and being much more persistent, after the heart is once under its influence.

So far as the results of these experiments allow me to form an opinion, convallamarin is a powerful and, under the proper circumstances, a very useful agent in the treatment of cardiac diseases, but it is certainly contra-indicated in advanced cases

in which the muscular structure of the heart has undergone degeneration and change. The varying results which clinical observations have given is to be accounted for by the fact that the peculiar morbid conditions under which convallaria is useful are not yet thoroughly understood. Not more than one or two full medicinal doses should be given daily on account of the cumulative action of the drug.

Before closing I desire to express my obligations to Prof. H. Newell Martin and Mr. H. H. Donaldson, to whose kindness and suggestions I am much indebted.

EXPLANATION OF PLATE VII.

All tracings to be read from left to right.

Fig. 1. Tracing taken by means of mercury manometer connected with the cardiac end of the outflow tube, in Experiment VI. The portion of the tracing reproduced in the figure commences before the heart had fully recovered under unpoisoned blood from the effects of a dose of carbolic acid, and continues until the normal beat is nearly regained.

Fig. 2. From the same experiment: shows recovery under atropinized blood from a dose of carbolic acid.

Fig. 3. Tracing taken with Roy's tonometer of the beat of the heart under a dose of convallamarin. To the left the tracing is normal; to the right the effect of the convallamarin in decreasing or almost abolishing the diastole of the heart is manifest.

THE ACTION OF INTERMITTENT PRESSURE AND OF DEFIBRINATED BLOOD UPON THE BLOODVESSELS OF THE FROG AND THE TERRAPIN.

By LEWIS T. STEVENS, A. B., Fellow of the Johns Hopkins University, and FREDERIC S. LEE, A. M., Graduate Scholar of the same.

A change in arterial pressure, produced by a drug, may be the result of its action, direct or indirect, upon the heart or the bloodvessels, or upon both. Evidently, before the cause of the change can be known, the specific effect of the drug upon the heart and upon the bloodvessels must be determined. The heart must be isolated from the bloodvessels of the whole body, placed under conditions which resemble, as closely as possible, the natural ones, and be subjected to the action of the drug. And, likewise, the bloodvessels must be made the single factor in the circulation and the effect on them be studied.

In 1882 a method was devised in this laboratory for determining the direct and indirect action of substances upon the bloodvessels of cold-blooded animals, and was used with success in a study of digitaline.[1] This method consisted in cutting out the heart, and inserting cannulas into the aortic trunks and the venae cavae. The aortic cannulas were connected with Mariotte's flasks placed at a certain height above the body of the animal, from which flasks liquid flowed into the arteries. This liquid, after completing the circulation through the capillaries and veins, was drained off from the venae cavae, and measured at definite intervals. After this venous outflow had become constant, circulating fluid plus drug was substituted for circulating fluid alone, and the action of the drug on the bloodvessels was determined from the change which took place in the amount of the outflow in a unit of time; a decreased outflow indicating increased resistance to the flow of the fluid through the vessels, *i. e.* a constriction of them, and an increased outflow, a dilation of them.

[1] Donaldson and Stevens, Journal of Physiology, Vol. IV, p. 165.

As circulating fluid, defibrinated blood first suggested itself, but it was found impossible, for some unknown reason, to obtain a flow of blood through the vessels. The cause of this was supposed to be a clogging of the capillaries with corpuscles. It was, therefore, found necessary to make use of some other liquid, and a .75 per cent. solution of sodium chloride was selected. The method, as it thus stood, was imperfect in several respects.

1. The force driving the liquid through the vessels was a constant pressure, while normally it is an intermittent one.

2. By watching the venous outflow, it can readily be determined whether a drug exerts a constricting or a dilating action upon the vessels; but the amount of the constriction or dilation, and the effect upon the pressure in the arteries, cannot thus be determined. Suppose, for example, that the Mariotte's flasks stand 12 cm. above the body of the animal, *i. e.*, that the force driving the liquid through the vessels is that exerted by a column of salt solution 12 cm. in height; that digitaline be given, and the effect obtained be a reduction of the venous outflow from 8 cc. per minute to zero; what is to be inferred? The conclusion to be drawn is not that the arterioles are constricted to complete closure, but simply that the resistance offered by the arterioles to the flow has been increased to such an extent that a force greater than that exerted by a 12 cm. column of salt solution is now required to drive liquid through them; but whether the pressure that is requisite to force through 8 cc. per minute will amount to 20, or even 60 cm. of salt solution, or whether the resistance has been increased to such an extent that under no pressure, not sufficient to burst the vessels, can liquid be forced through, is not thus to be determined. It is, therefore, evident that by this method only a portion of the whole effect of a drug can be determined; but that this portion increases as the Mariotte's flasks are raised higher above the animal. But on raising the flasks the outflow gradually increases, and for constant pressures above a certain limit becomes abnormal. Hence, only by making the inflow fixed and independent of the resistance offered by the vessels, and by knowing the pressure which actually exists in the arteries both before and during the action of the drug, can the whole effect of the drug on vascular constriction be determined.

3. As circulating fluid, a liquid was used which does not contain nutritive material for the use of the tissues of the bloodvessels, but, on the contrary, being a good solvent of certain varieties of albumen, rather extracts material from the tissues, and consequently tends to put them in a condition which approaches nearer and nearer to exhaustion as the experiment proceeds. It is true that salt solution does keep the vessels of the frog and of the terrapin in a nearly normal condition for a couple of hours, but it is desirable that a really nutritive liquid be used.

It was the object of the present investigation to correct these defects in the method; to substitute remittent for constant pressure; to so arrange the experiment that the whole effect of the drug could be accurately observed; and to find, if possible, a better circulating fluid. The work led to results which were quite unexpected. These can be best stated under three heads:

I. The method.
II. The action of intermittent pressure upon the bloodvessels.
III. The action of defibrinated blood upon the bloodvessels.

The Method.

To imitate the natural conditions as closely as possible, the heart of the animal was replaced by an instrument, which, like the heart, forced intermittently into the arteries, at a uniform rate, equal amounts of the circulating fluid. The apparatus is simple in principle and construction. It consists of a horizontal hammer-shaped brass bar about 9 inches long and so fixed that it can move in a vertical plane about an axis situated 3 inches from the end of the handle. A cross piece of soft iron is fastened to its middle just above the cores of a double electro-magnet placed vertically, but is kept off them by a spring attached to the handle-end of the bar. The instrument is placed between Mariotte's flasks containing the circulating fluid and the bloodvessels of the animal, so that the rubber tubing, leading from the former to the latter, comes directly under the hammer portion of the brass bar. The electro-magnet is placed in an electrical circuit with a clock, the pendulum of which opens and closes the circuit regularly and at a desired rate; every time the circuit is closed the bar is brought down to the electro-magnet, and the

head of the hammer compresses the rubber tubing. On each side of the portion of tubing which passes under the hammer is placed an Ewald's valve,[1] and these two valves are so arranged that the liquid, which is forced out of the tubing at each downward stroke of the hammer, can pass in but one direction, into the arteries of the animal. The amount of fluid thus sent into the vessels by each beat of the "artificial heart," is not at all affected by the resistance which the bloodvessels may offer to its passage through them, but depends upon the amount of compression which the rubber tubing undergoes. This latter can easily be increased or diminished, and, after being once regulated, remains constant throughout the experiment. The height of the bottom of the air tube of each Mariotte's flask above the "artificial heart," is fixed at 10 cm. The arterial pressure, which is taken from one of the smaller arteries and is recorded on smoked paper by means of a mercury manometer, depends upon the amount of fluid forced into the arteries at each beat, upon the rate at which the artificial heart is beating, and upon the vascular resistance. The first two factors remain constant throughout an experiment, the rate being in all our experiments 30 beats per minute, which is about the normal pulse rate of the frog and the terrapin at ordinary temperatures; the vascular resistance is, therefore, the only variable factor, and any variation which may occur in this makes its impression upon the arterial pressure and is recorded. The rest of the method remains as it originally was. The fluid after completing its circulation is siphoned off from the venous sinus, or from the venae cavae, through one or more large cannulas. The end of the rubber tubing, attached to the venous cannulas, is fixed at 3 cm. below the level of the venous sinus, the object of this being to prevent the fluid from collecting in the veins and hindering free circulation. The outflow is collected and measured at definite intervals, chiefly for the purpose of detecting leaks which may be produced by a rapid and great rise of arterial pressure. With salt solution as a circulating fluid, under a high pressure, the amount of the outflow is somewhat diminished in consequence of extravasation.

As regards the operation upon the animal, if the brain and

[1] Ewald u. Kobert, Pflüger's Archiv, Vol. XXXI, p. 160.

spinal cord are to be included in the circulation, the frog or terrapin is curarized and then bled by making incisions in the auricles and ventricle; the pulmonary artery is ligated; cannulas are placed in the aortic trunk or trunks, and in the central end of one carotid artery (frog), or of one brachial (terrapin). The ventricle is cut away, and, in the case of the frog, a cannula is inserted into each of the three venae cavae; in the terrapin one large cannula is tied directly into the venous sinus. The animal is then placed in a square case; connections are made between the rubber tubing leading through the artificial heart from the supply-flasks and the aortic cannulas; between the carotid, or brachial, cannula and the Hg manometer; between the venous cannulas and the outflow tube; a thermometer is placed beside the animal, the case is covered, and the artificial heart is regulated and set to work. As a rule, the drum is made to revolve slowly, and a continuous tracing of the pressure is taken for the whole experiment. The effect upon the bloodvessels of a drug, or of changed conditions, is not studied until the pressure has become constant and the outflow free and steady.

If the central nervous system is to be excluded and the direct effect of a substance, or of changed conditions, upon the blood-vessels to be determined, the brain and spinal cord of the animal are destroyed, and the abdominal viscera and hind legs only are kept in the circulation.

As circulating fluid, .75 per cent. sodium chloride solution is still used, for we found that neither defibrinated blood nor serum, even in very diluted condition, could, for reasons which are given under the third division of this article, be employed. However, with salt solution circulation can be kept up for at least two hours, without producing any very great changes in the pressure, or in the elasticity of the vessels, as shown by the height of the pulse curve, and probably without seriously affecting their contractility. The changes in pressure and in the elasticity of the vessels are shown by the following table.

TABLE I.

Experiment VI. January 10th, 1884. Terrapin, weight 1000 grammes, curarized. Duration of experiment, 2 hours 35 minutes.

Time. P. M.	Temperature in degrees C.	Pressure in brachial artery in mm. Hg.	Height of pulse curve in mm. Hg.	
1h. 15m.				Terrapin in case and circulation begun.
29		12.8	3.2	
2.10	20.8	11.0	3.0	
.20		9.7	2.8	
.30		10.2	3.0	
.40		10.3	3.0	
.50	20.6	9.7	2.8	
3.00		10.3	2.8	
.10		10.0	3.0	
.20		9.5	2.6	
.30		9.0	2.5	
.40		8.8	2.0	
.50		9.0	2.1	

The tracing (Fig. 1) obtained under salt solution shows the pressure to be nearly uniform, marked, however, by slight rhythmical rises and falls; which changes are sometimes comparatively rapid in rate and considerable, sometimes slow and slight. That these changes are due, not to alternate constriction and dilation of the arterioles, but rather to rhythmical changes in the elasticity of the vessel-walls, is indicated by the fact that, as the accompanying tracing shows, the lower ends of the separate pulse curves remain always at the same distance above the base line, while their heights rhythmically vary.

The Action of Intermittent Pressure upon the Bloodvessels.

For theoretical reasons already stated, the pressure existing in the arteries was changed from a constant to a rhythmically varying one. Our experiments actually show that this latter kind of pressure is not only desirable, but that it is necessary if the bloodvessels are to be kept in their normal, tonic condition.

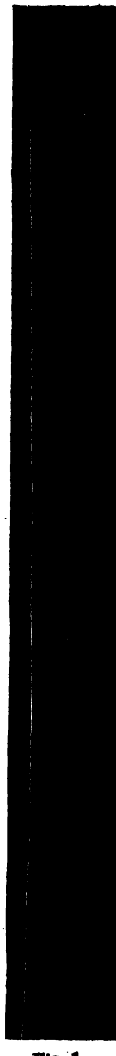

Fig. 1.

Comparison of our experiments on intermittent pressure with those of a similar kind on constant pressure made during the previous year by Donaldson and one of us[1] gives indications of a specific action of rhythmically interrupted pressure upon the smaller arteries or upon arteries large and small. The following Table II shows this. Unfortunately the terrapins used last year, though of the same species, *Pseudemys rugosa*, were larger than those used in our experiments; but to reduce the difference which this would make in the amount of venous outflow, to as near zero as possible, experiments made last year upon the six lightest terrapins are compared with ours upon the six heaviest. The first division of the table contains results obtained on curarized terrapins, when their arteries were supplied with liquid under constant pressure; the second division, results of the same kind upon curarized terrapins when liquid was supplied under an intermittent pressure;[2] the third division, results on terrapins whose brains and spinal cords were destroyed and to whom liquid was supplied under intermittent pressure. In each division the first column contains the weights of the animals in grammes; the second, the pressures[3] in the arteries at the commencement of the experiment, expressed in cm. of water; and the third, the venous outflow per minute at the same time.

TABLE II.

I. Terrapins curarized.			II. Terrapins curarized.			III. Terrapins with brain and spinal cord destroyed.		
Constant inflow.			Intermittent inflow.			Intermittent inflow.		
Weight.	Press're	Outflow.	Weight.	Press're	Outflow.	Weight.	Press're	Outfl'w
1165	12.0	6.5cc.	1125	39.2	5.4cc.	1125	25.1	2.2cc.
1250	14.0	7.8	1235	28.6	2.2	1675	11.5	3.0
1650	14.0	4.5	1225	20.1	5.0	1560	17.0	5.4
1800	14.5	15.0	1060	22.0	4.1	1195	16.0	6.6
1818	10.5	11.6	1325	27.2	4.8	1150	16.8	3.4
1770	17.0	12.6	1200	30.3	2.6	...	...	...

[1] *Op. cit.*

[2] For easy comparison, the pressures are given in cm. H_2O, instead of mm. Hg.

[3] These values given for the constant inflow do not accurately express the

The special point to be noticed in the above table is the low constant pressure and large outflow as compared with the high rhythmically varying pressure and small outflow. For example, from a terrapin weighing 1250 grammes, a venous outflow amounting to 7.3 cc. per minute was obtained, when the pressure was constant and amounted to 14 cm. of water; while from another terrapin of about the same weight, an outflow of only 2.2 cc. per minute was obtained, when the pressure was remittent; yet despite this small outflow (and corresponding inflow) the pressure amounted to 28.6. cm. water. Or to state it differently, it required a constant pressure of only 14 cm. water to drive through the capillaries 7 cc. of fluid per minute, but an intermittent pressure whose mean was 29 cm. water to drive through but 2 cc. per minute.

Experiments on frogs show the same peculiarity.

TABLE III.

FROGS CURARIZED.

Constant Inflow.			Intermittent Inflow.		
Weight.	Pressure.	Outflow.	Weight.	Mean Pressure.	Outflow.
300 grms.	17.0	8.1	145	13.9	4.8
250	14.0	12.6	165	18.4	1.8
275	11.0	7.1	315	11.6	3.5

Now the cause of this must be either physical or physiological. If physical, then the same observation can be made on an artificial schema. It is, of course, hardly possible that the resistance offered by capillaries to the flow of fluid through them, in consequence of the friction of it against their walls, can be increased by changing the character of the driving force; nevertheless, to put aside this bare possibility, an experiment was made using a system of glass capillaries instead of those of the terrapin. The result was what was expected, namely, that for rigid capillary

arterial pressures, since they denote in each case the difference between the level of the lower end of the air tube of the Mariotte's flasks, and of the animal. The actual arterial pressures were of course less than these values, but exactly how much less there is no means of determining.

walls it makes no difference in the outflow or in the pressure whether the driving force be constant or interrupted, provided its mean amount be the same.

It would, therefore, appear that a varying driving force exerts a peculiar action upon the bloodvessels, in consequence of which a much smaller amount of liquid is needed to maintain a given arterial pressure, than when the force is a constant one. That this action is not exerted through the agency of the central nervous system is shown by a consideration and comparison of the third with the first division of Table II. We see that when all vasomotor influences, originating centrally, are excluded, the arterioles are still able, under the stimulus of an interrupted force, to oppose so much resistance to the passage of the liquid as to keep up a comparatively high pressure in the larger trunks with a small supply of liquid.

We next endeavored to determine the difference in effect, if any, between constant and interrupted forces upon the bloodvessels of the same individual. The fluid was first allowed to flow into the vessels from Mariotte's flasks, and the outflow per minute and the constant pressure in the arteries were determined. Then, by the turning of two stopcocks, the inflow was changed from a steady to a remittent one, and the outflow and pressure measured under this change of conditions. Such experiments have been made only on terrapins, but we feel justified, after having obtained the results given in Table III, to apply the conclusions drawn from the terrapin experiments to frogs also.

The following table gives the details of one of such experiments.

TABLE IV.

Experiment III. April 29, 1884. Terrapin, weight 1400 grammes. Brain and spinal cord destroyed. Duration of experiment, 2 hours 45 minutes.

Time. P. M.	Arteri'l Press're in mm. Hg.	Venous Outflow in cc. per min.	
12h.20m.			Terrapin in case.
1.05	12.4	2.8	Constant pressure from 12.30 to 1.30 P. M.
10	10.1	3.1	
15	10.8	2.9	
20	10.8	3.5	
25	10.8	4.3	

Time. P. M.	Arterial Press're in mm. Hg.	Venous Outflow in cc. per min.	
1.30	10.8	5.5	Remittent pressure from 1.30 to 1.52.
35	18.3	3.7	
40	16.5	3.2	
45	18.4	3.7	
50	21.5	3.5	Constant pressure from 1.52 to 2.40.
55	12.5	2.2	
2.00	12.4	1.1	
05	11.8	.6	
10	11.9	.5	
15	11.5	.7	
20	10.8	2.6	
25	10.6	4.3	
30	10.5	5.1	
35	10.5	6.2	
40	10.5	7.1	Remittent pressure from 2.40 to 3.05.
45	11.0	3.1	
50	11.4	3.2	
55	13.0	3.4	
3.00	14.4	3.5	
05	16.0	3.3	Experiment ended.

In this experiment when the force which drives the fluid through the animal is constant, an inflow (equal to outflow) of 5.5 cc. per minute is needed to keep up an arterial pressure of 10.8 mm. Hg. If this force be now made intermittent, a much higher pressure, about 19 mm. Hg, can be maintained with even a smaller supply of liquid, namely, 3.5 cc. per minute. This is the result that is invariably obtained when an interrupted instead of a constant force is employed, a great increase of arterial pressure with the same or even a smaller supply of circulating fluid; and this result clearly points to a specific action of the intermittent force on the bloodvessels, very probably on the muscular arterioles especially, in consequence of which increased resistance is offered to the flow of circulating fluid. The phenomenon observed in changing back from an intermittent to a constant pressure also points directly to the existence of a constricting action by the former. The constant force (represented by a column of salt solution equal in height to the difference in level of the lower end of the air tube in the Mariotte's flasks and of the animal) is not great enough to overcome the increased resistance, and as a result the inflow stops, the outflow falls rapidly to zero, and only after the arterioles have slowly relaxed

is the constant force great enough to overcome the resistance and circulation is resumed.

From the results of special experiments also, we are led to the conclusion, that *a rhythmically interrupted force, applied to the bloodvessels of the frog and the terrapin, through the medium of a circulating fluid, exerts a special action upon them, in consequence of which a constriction of them takes place; this constriction, however, probably never exceeds that amount, which one calls normal and tonic.*

The Action of Defibrinated Blood upon the Bloodvessels.

Defibrinated blood is an undesirable liquid for artificial circulation, because with it it is impossible to obtain a uniform pressure and outflow. If a uniform pressure and outflow be obtained with salt solution, and then whipped blood be allowed to enter the vessels, the pressure rises very rapidly to an amount at least twice, generally three or four times, the pressure under salt solution; and the amount of outflow falls rapidly towards zero. To bring about such results a surprisingly small amount of blood is needed, namely, for the frog or the terrapin, 15 to 30 cc. That this change is not caused by a clogging of the capillaries with corpuscles is indicated by the rapidity with which the change is produced, and is proved by the fact that blood diluted ten or more times with salt solution, or even greatly diluted serum, has the same effect as undiluted blood. These statements are confirmed by the two tables which follow. Table V shows the effect upon the terrapin's blood-pressure of both defibrinated blood and serum taken from an animal of a different species; Table VI the effect of terrapin's blood and serum upon a terrapin's arterial pressure. From these two tables, and others which follow, the measurements of the outflow are omitted, because not so definite conclusions can be drawn as to the action of a substance upon the vessels from the changes in outflow as from the changes in pressure. In every case serum was obtained by allowing blood to clot and to stand.

TABLE V.

Experiment II. December 20, 1883. Terrapin, weight 850 grammes. Curarized. Circulating liquids, .75 per cent. NaCl solution, defibrinated dog's blood, and dog's serum (obtained from clot upon 3 hours standing). Duration of experiment, 3 hours 20 minutes.

Time. P. M.	Temp. in deg. C.	Height of pulse curve in mm. Hg	Arterial Press're in mm. Hg.	
1h. 00m.				Terrapin in case, and circulation of salt solution begun.
25			15.0	
30	20.1	3.7	13.0	
35		3.3	16.5	
40		3.6	14.0	
45		2.7	15.5	Diluted blood on (1 part blood to 5 parts salt solution). Blood reached vessels at 1.46½.
48		3.0	25.5	Blood off, 15 cc. having been run through; salt solution on.
51		3.5	54.5	
54		3.4	41.5	
57		3.4	44.5	
2.00		2.5	35.5	
05		1.8	25.0	
15	20.4	2.5	21.5	
25		2.0	18.0	At 2.27 diluted serum on (1 part serum to 5 parts salt solution). At 2.28½ serum first reached the bloodvessels.
30		1.6	24.5	
33		2.5	35.5	Serum off, 30 cc. having been run through; salt solution on.
36		3.4	47.0	
40		2.0	34.0	
50		1.7	27.5	
3.00	20.5	1.4	26.5	
10		2.0	18.0	Diluted blood on (1 part blood to 10 parts salt solution).
13		2.0	19.0	At 3.11½ blood reached vessels.
19		1.2	25.5	
22		1.4	26.5	At 3.21 blood off, 50 cc. having been run through; salt solution on.
25		1.4	24.0	
35		1.6	18.5	
45	20.6	1.9	17.0	Diluted serum on (1 part serum to 10 parts salt solution).
48		1.9	17.5	At 3.46½ serum reached vessels.
51		1.0	25.0	
54		1.4	27.0	
57		.8	31.0	
4.06			33.0	At 4.03 serum off, 90 cc. having been run through; salt solution on. Slight œdema.
10			31.0	
20		.9	22.0	Experiment ended.

TABLE VI.

Experiment V. January 9, 1884. Terrapin, weight 655 grammes. Curarized. Circulating liquid, terrapin's serum, alkaline. Duration of experiment, 2 hours 36 minutes.

Time. P. M.	Temperature in deg. C.	Height of pulse curve in mm. Hg.	Arterial pressure in mm. Hg.	
3h. 20m.				Terrapin in case, and circulation of salt solution begun.
35	19.5	2.3	26.0	
55		2.2	22.0	
4.05		2.1	22.8	At 4.07 dilute serum on (1 part serum to 1 part salt sol.)
10		1.9	22.5	At 4.11 serum reached bloodvessels.
13		3.3	40.0	Serum off, 12 cc. having been run through; salt solution on.
16		2.0	46.7	
19		2.7	62.7	
22		2.7	63.7	
25		2.5	65.5	
28		2.0	61.0	
40		1.5	38.2	
50	19.6	1.8	31.2	
5.00		1.1	24.0	At 5.01 dilute serum on (1 part serum to 5 parts salt solution).
04		1.0	23.8	At 5.06 serum reached vessels.
07		1.6	31.0	
10		2.0	39.0	Serum off, 20 cc. having been run through; salt solution on.
15	20.0	1.9	33.5	
25		1.4	26.0	
35		1.1	23.0	At 5.38 dilute serum on (1 part serum to 10 parts salt solution).
40		1.2	23.3	
44		1.0	25.0	At 5.48 serum reached vessels.
47		1.3	28.6	
50		1.6	32.5	
53		1.4	32.2	
56		1.6	32.2	Serum off, 35 cc. having been run through; experiment ended.

We conclude from these and other similar experiments that defibrinated blood and serum constrict the bloodvessels—probably the arterioles—and thus raise pressure in the arteries. Blood and serum appear also to decrease the elastic extensibility of the vessel walls, as is seen from the height of the pulse curve, which gradually decreases throughout the experiment (see especially Table V); this decrease in elasticity as a result of the action of defibrinated blood will be found more prominent when we come

to compare the effects of normal unclotted blood with those of defibrinated blood. Salt solution similarly, but not so rapidly, diminishes the elasticity. That the above results are not due to any action of the main nerve centres is shown by the following table, which gives the details of an experiment upon a terrapin whose brain and spinal cord had been destroyed.

TABLE VII.

Experiment XXIII. March 13, 1884. Terrapin, weight 1200 grammes. Brain and spinal cord destroyed. Circulating liquid, terrapin's serum. Duration of experiment, 57 minutes.

Time. A. M.	Temperature in deg. C.	Height of pulse curve in mm. Hg.	Arterial pressure in mm. Hg.	
11h.05m.	18.4			Terrapin in case, and circulation of salt solution begun.
31		2.7	27.4	
34		3.0	28.0	
37		2.8	29.4	
40		3.0	27.0	
43		3.9	29.6	Dilute serum on (1 part serum to 2 parts salt solution).
46		3.0	27.5	Serum reached vessels.
49		4.0	42.5	
52		3.2	44.2	Serum off, 20 cc. having been run through; salt solution on.
55		2.6	42.1	
58	18.8	2.5	30.4	
P. M.				
12.02		2.1	25.2	Experiment ended.

As far as we know a direct constricting action has never before been claimed for defibrinated blood or serum, although several observers have noticed a rise of pressure or a decrease in outflow when these liquids were used for artificial circulation. Mosso,[1] in the account of his plethysmographic work upon the kidney, describes the outflow as great when the circulation of defibrinated blood begins, but falling in a few minutes to a small fraction of its original amount, and ascribes the phenomenon to a change in the elasticity of the vessel walls, without attempting to explain

[1] Mosso, Von einigen neuen Eigenschaften der Gefässwand, Ludwig's Arbeiten, Vol. IX, 1874, p. 156.

the character of the change. Bernstein[1] observed a similar decrease in outflow in artificial circulation of defibrinated blood through the dog's leg, and is inclined to believe in a contraction of the muscles of the vessel walls as one of its causes. Ludwig and Schmidt,[2] in their paper on the gaseous exchange in resting and working dog's muscle, through which blood was kept circulating for hours, make the following statement: "Ein Druck von bestimmter Höhe (40 bis 60 mm. Hg) der in den ersten 30 bis 60 Minuten ein bestimmtes Volum Blut in der Zeiteinheit durch den Muskel treibt, muss in der dritten und vierten Stunde des Versuches oft verdoppelt werden (100 bis 150 mm. Hg), wenn er auch jetzt die ursprüngliche Menge von Blut durchführen sollte"—an observation which points directly to an active constricting action of defibrinated dog's blood. Cohnheim[3] in recommending the transfusion of blood into an artery instead of a vein, states that it is "impossible to inject the defibrinated blood into the peripheral end of the artery, because the peripheral arterial branches constrict against the foreign blood with such energy that it requires considerable force to overcome the resistance, a force sufficient to burst the arterial walls; but such a great resistance is not met if the blood be injected into the central end," for the simple reason, we think, that the same amount of defibrinated blood is quickly distributed over a greater vascular area. Köhler[4] also makes the same statement. Finally, Worm-Müller[5] observed that the immediate effect of injecting 40 cc. of defibrinated dog's blood into a dog is a rise of pressure; no doubt, a result of injecting into the jugular vein. This initial rise is followed by a second rise, which, however, is very irregular in appearance, and varies from 1 to 4 cm. Hg. He simply states that the second rise appears nearly every time, but does not attempt to explain it. It can hardly be an effect of the increase in the volume of blood due to the injection.

[1] Bernstein, Versuche zur Innervation der Blutgefässe, Pflüger's Archiv, Vol. XV, 1877, p. 575.

[2] Ludwig u. Schmidt, Das Verhalten der Gase, welche mit dem Blut den reizbaren Säugethiermuskel strömen. Berichte der kön. sächs. Ges. d. Wiss. 1868, p. 26.

[3] Cohnheim, Allgemeine Pathologie.

[4] Köhler, Ueber Thrombose u. Transfusion, etc., Dorpat, 1875.

[5] Worm-Müller, Arb. aus d. physiol. Anstalt zu Leipzig, VIII, p. 159.

Now the question comes up for consideration—what is it in the whipped blood which produces the marked effect which we have observed? Blood in the condition in which it exists in the vessels of the living body can not have such a constricting action upon the vessels, or circulation would be impossible. We have attempted to prove by experiment the truth of this *à priori* statement and to show that "living" blood is neutral to the vessels. On account of the rapidity with which degenerative changes follow the drawing of blood, experimenting with "living" blood is an exceedingly difficult matter. We have employed two different methods of work. The first was to draw the blood from one terrapin directly into a funnel from which a short inflow tube ran to the artificial heart and thence into the body of the terrapin whose bloodvessels were to be experimented upon, the object being to so hasten the introduction of the blood into the vessels that not sufficient time should elapse for the degeneration to set in. But this method proved quite unsatisfactory, for very soon after the blood had passed into the terrapin, the supply in the funnel would begin to clot. The results of the single experiment performed in this way, though not at all definite, are nevertheless instructive. The blood for the first six minutes produced no practical change in pressure; after this time the pressure went up, not suddenly, as it does with defibrinated blood, but gradually.

The second method consisted in retarding the appearance of the degenerative changes by cooling the blood; but before this method could be used, the effect of heat and cold upon the bloodvessels had first to be determined. In the experiments on this point, heated salt solution was run through, then cold salt solution, and this was repeated several times, while a continuous tracing of pressure was taken throughout the whole experiment. The range in the temperature of the liquid just before it entered the vessels of the animal was in one experiment 7.4° to 20.3° C., in another 11.8° to 40° C.; but the highest extreme was, of course, somewhat lowered by the time the fluid reached the arterioles; the actual variations of temperature must, however, have been sufficient to affect the vessels, if they were capable of being affected by heat and cold. It is hardly necessary to give a table showing the results of such an experiment; it is suffi-

cient to simply state that the arterial pressure is not at all affected by such changes of temperature, apart from that small change produced by a slight alteration in the elasticity of the vessels, the individual pulse-curves being higher for heated than for cold salt solution. To return to the blood experiments. The method of procedure was as follows: A terrapin was prepared in the usual way for an experiment, and the circulation of salt solution carried on until the pressure and the outflow had each become uniform. Another terrapin was then bled directly into a funnel surrounded by ice, and from this, as in the first method, a short inflow-tube ran through the artificial heart to the prepared animal.

The following table gives the results of such an experiment.

TABLE VIII.

Experiment XXXVIII. May 12th, 1884. Terrapin, weight 1245 grammes. Brain and spinal cord destroyed. Duration of experiment, 1 hour 54 minutes.

Time. A. M.	Temperature in deg. C.	Height of pulse curve in mm. Hg.	Arterial press're in mm. Hg.	
11h.45m.				Terrapin in case, and circulation of salt solution begun.
57		1.0	12.0	
12 M.		1.0	11.6	
P. M.				
03		1.2	10.0	
06	20.4	1.8	10.1	
09		2.2	10.6	
12		2.5	11.8	Unclotted terrapin's blood on.
15		4.0	14.4	
18		3.8	14.4	
21		2.4	13.4	
24		3.1	12.5	Blood off, 65 cc. having been run through; salt solution on.
27		4.5	13.4	
30		3.2	12.8	
33		4.1	13.0	
36		4.0	12.6	
39		4.2	13.0	
42	20.5	4.8	14.0	From 12.24 to 1.06 marked rhythmic variations in height of pulse.
45		4.8	12.5	
48		4.2	14.0	
51		3.9	13.0	

Time P. M.	Temperature in deg. C.	Height of Pulse curve in mm. Hg.	Arterial Press're in mm. Hg.	
12.54		4.6	13.7	
57		4.1	13.3	
1.00		4.8	13.5	
03		5.0	14.0	
06		5.1	15.0	
09		3.5	14.7	Defibrinated terrapin's blood on.
12		1.4	11.6	
15		1.2	12.1	At 1.14 blood off, 40 cc. having been run through ; salt solution on.
18		1.1	23.1	
21		2.1	22.1	Pressure irregular.
24		3.1	21.7	
27	20.6	2.5	15.6	
30		2.0	12.0	
33		1.8	11.3	
36		2.0	12.2	
39		1.8	11.4	Experiment ended.

Although the normal unclotted blood was allowed to circulate through the vessels for 12 minutes, yet no effect was produced beyond an increase in the height of the pulse-curve and a consequent slight rise of mean pressure. We may, therefore, conclude that normal "living" blood tends to put the vessels into their normal condition of elasticity and tonicity, but exerts no effect beyond this. The action of defibrinated blood on the elasticity of the vessels is also clearly shown by this experiment. Circulation of normal blood for 12 minutes is sufficient to put the arterioles in a healthy condition; the height of the pulse-curve is increased to 4 or 5 mm. Hg, and remains at that height during the succeeding circulation of salt solution. If defibrinated blood be now turned on, the pressure rises to 23 mm. Hg, and the height of the pulse-curve falls rapidly from 5 to about 2 mm. Hg. It would have been interesting to have hindered the destructive changes in the blood by peptone and then to have determined the effect of peptonized blood, but a lack of terrapins prevented our using the peptone we had prepared for this purpose.

We are now forced back to our original question—what is it in whipped blood which produces the effects we have observed? Since normal "living" blood does not cause them, we must believe that the active agent is something not present in "living" blood,

but existing in defibrinated blood; and our attention was immediately directed to those substances which are set free before and during the process of coagulation. By the breaking down of the colorless corpuscles, paraglobulin and fibrin ferment are formed. During coagulation, the fibrinogen normally present in the blood, and possibly some of the paraglobulin, disappear. The fibrinogen breaks down, yielding fibrin and, according to Hammarsten[1] and Howell,[2] a certain other albuminous substance. Defibrinated blood contains this latter substance, a greater amount of paraglobulin than is present in "living" blood, and finally fibrin ferment. The comparatively little known albuminous substance produced by the decomposition of the fibrinogen may be left out of consideration, for we have found that plasma which has stood for several hours surrounded by ice, will, at the end of that time, though unclotted, affect the bloodvessels similarly to defibrinated blood and serum. The cold has presumably not entirely prevented the dissolution of the colorless corpuscles, although it has prevented coagulation. Paraglobulin is normally present in the blood in considerable quantities, and it is very improbable that the slight increase in its amount, caused by the destruction of the colorless corpuscles, would change the blood from a neutral liquid to one powerfully active as regards vascular contraction. Fibrin ferment is then the only known substance left to be considered. Experiments with it were performed upon terrapins with terrapin's fibrin ferment. Solutions of the ferment were obtained by allowing terrapin's defibrinated blood, or serum, to stand under 20 times its volume of 90 per cent. alcohol for 4 weeks, during which time the flask containing the mixture was well shaken daily, and the alcohol renewed weekly. At the end of this time the precipitate was collected on a filter, dried over sulphuric acid, and ground to a powder. A solution was made by allowing the powder to stand under distilled water over night. In this way a ferment solution was obtained, which on boiling became not at all, or but slightly, turbid, was perfectly neutral in reaction, and possessed very active fermentative powers. To this solution enough common salt was added to make it a 0.75 per

[1] Hammarsten, Pflüger's Archiv. XXX, p. 437.
[2] Howell, Studies from Biol. Lab., J. H. U. Vol. III, p. 63.

cent. salt solution. When this was circulated through a terrapin, a constriction of the vessels, equal in amount to that produced by defibrinated blood, was effected, as evidenced by the following table. Ferment solution No. 1 was obtained from terrapin's serum. It was perfectly colorless, neutral in reaction, and on boiling gave the slightest trace of a turbidity. Ferment solution No. 2 was obtained from terrapin's blood, was slightly brown in color, perfectly neutral, and on boiling gave not the slightest trace of a precipitate.

TABLE IX.

Experiment XXIX. April 24, 1884. Terrapin, weight 1115 grammes. Brain and spinal cord destroyed. Duration of experiment, 1 hour 21 minutes.

Time. A. M.	Temperature in deg. C.	Arterial press're in mm. Hg.	
11h.15m.			Terrapin in case, and circulation of salt solution begun.
30		16.0	
33		18.7	
36		16.7	
39		15.4	
42		16.2	Ferment solution No. 2 on.
45		17.7	
48	20.3	46.3	At 11.47 ferment solution off, 30 cc. having been run through; salt solution on.
51		41.2	
54		33.7	
57		31.5	
12 M.		27.0	
P. M.			
03		24.0	
06		19.8	
09		19.7	
12		19.2	Ferment solution No 1 on.
15		26.0	
18		35.3	
21		27.0	At 12.19 ferment solution off, salt solution on.
24		25.3	
27		19.7	
30	20.1	20.2	
33		21.3	
36		19.8	Experiment ended.

From the results of this and of other experiments, we conclude that *fibrin ferment is the active substance in defibrinated blood which causes the constriction of the arterioles.*

It now remains for us to state the conclusions which we deduce from our work upon blood.

1. Normal "living" blood puts the vessels into their tonic condition, but exerts no effect beyond this.

2. Defibrinated blood powerfully constricts the vessels, and also appears to diminish their elasticity.

3. The chief, if not the only, constituent of defibrinated blood which exerts this influence is fibrin ferment.

4. Changes of temperature of the circulating liquid exert no influence upon the vessels, beyond a slight change in their coefficient of elasticity.

This article is now issued as the result of the joint labors of its two authors. The work is obviously incomplete in several respects; the effect of periodic variations of rhythm having different rates needs examination; and also the effect upon the elastic coefficient of isolated bloodvessels of the different liquids used for circulation in the experiments described above. It seemed, nevertheless, desirable to publish the results obtained up to the present, because, on account of the intended absence of one of the writers from Baltimore during the coming year, the continuation of the investigation cannot be a conjoint labor.

We desire here to express to Professor H. Newell Martin our sincere thanks for his kindness and his many suggestions to us.

THE CRANIAL MUSCLES OF AMIA CALVA (L), WITH A CONSIDERATION OF THE RELATIONS OF THE POST-OCCIPITAL AND HYPOGLOSSAL NERVES IN THE VARIOUS VERTEBRATE GROUPS.

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The position intermediate between the more typical Ganoids and the Teleöstei, usually assigned to *Amia*, suggested the desirability of investigating the relations of its cranial musculature, my expectations being that some important facts bearing on the origin of the comparatively specialized muscles of the Teleosts would thereby be brought to light. The results have amply fulfilled the expectations, *Amia* presenting a most interesting intermediate arrangement between that seen in *Acipenser* and that of a typical Teleost. The second portion of this paper, dealing with the distribution of the three nerves passing out from between the partially aborted vertebrae which lie immediately behind the true occipital region, and with the question as to their homologues in other vertebrate groups, will, it is hoped, aid materially in clearing up their true relationships.

For the greater portion of the material upon which I worked, consisting of three admirably preserved alcoholic specimens, I am indebted to Professor B. G. Wilder of Cornell University, who generously and with his characteristic disinterestedness placed them at my disposal. I desire here to express my most sincere thanks, both to him and to Professor Gage, for the assistance thus afforded.

Part I.

THE CRANIAL MUSCULATURE.

It will be convenient, on account of the intimate relations between some of the muscles which have quite distinct functions in higher forms, to consider the cranial muscles according to their nervous supply.

A.—*The Trigeminal Muscles.*

The *Adductor mandibulæ* is a large strong muscle, consisting of two almost distinct parts. The first, largest, and most superficial of these (Pl. X, Fig. 1, AM[1]) arises from under surface of the lateral portions of the postfrontal[1] and parietal above, and from the preoperculum, hyomandibular and quadrate behind and below. It is covered in front and above by the large postorbital, and traversing it from its anterior edge on a level with the eye downwards and backwards, is the *R. inf. max. Trigemini.* Its fibres are directed downwards and forwards, converging towards the mandible, and are inserted into its coronoid process and the adjacent parts, and into Meckel's cartilage.

On removing this superficial portion the second (Fig. 1, AM[2]) is exposed. It arises from the hyomandibular and quadrate, but mainly from the metapterygoid and the surface of a dense membrane stretched between the hyomandibular and metapterygoid. Its fibres also converge forwards and downwards, and unite to a broad tendon, which, diminishing in size, and receiving a tendon from two portions of the Lev. arc. pal., is inserted into the inner surface of the mandible, *i. e.* into the articulare.

The *Levator arcûs palatini* consists of several portions, certain of them bearing very little resemblance to any of the parts which can be referred to that muscle in Teleosts hitherto investigated. One portion is, however, comparable to the muscle recognized by that name in *Amiurus*,[2] for instance, although even here a slight expansion of the designation is necessary. This part (Fig. 1, LAP[1]) arises from the postfrontal and epiotic, together with the under surface of the membrane bones above them, and from the prootic region. Its anterior fibres are directed downwards to be inserted into the anterior edge of the metapterygoid, curving round somewhat to reach its anterior surface, while the more

[1] In designating the bones I have employed the terms used by Bridge in his paper entitled *The Cranial Osteology of Amia Calva*, Jour. of Anat. and Phys., Vol. XI.

[2] J. Playfair McMurrich. *The Myology of Amiurus Catus.* Proceedings of Canadian Inst. Toronto. Vol. II, 1884.

posterior fibres are inserted into its upper border, and those lying most posteriorly are directed backwards, passing under the upper extremity of the preoperculum so as to be inserted into the operculum, thus fulfilling the function of the separate *Dilatator operculi* of the Teleostei.

The second portion (LAP^2) lies behind this, and in reality behind the plane of the metapterygoid. It is a thick, stout muscle, arising from the prootic and postfrontal regions, and at first passing almost directly forwards, but on reaching the level of the anterior border of the metapterygoid it turns downwards, and, becoming tendinous, unites with the broad tendon of the second portion of the Add. mand. What may be termed the third portion (LAP^3) is really a division of the second, a separation of them above being impossible. Certain of the fibres which arise from the postfrontal almost immediately separate themselves from the remainder of the muscle, and, passing downwards and slightly forwards, are inserted into the inner surface of superficial portion of the Add. mand. where it passes behind the coronoid process of the mandible.

The fourth portion (LAP^4) arises in front of the eye from the prefrontal region. It forms a round belly, contracting below into a long, narrow tendon which unites with that of the second portion, and fuses with it, with the broad tendon of the deeper portion of the Add. mand.

The fifth portion (LAP^5) seems to have little connection with the other parts of the muscle, and perhaps ought not strictly to be included as one of its parts, but, since it belongs to the group supplied by the Trigeminus, it seems most convenient to describe it here. It arises from the palatine bone, forming at once a stout, round belly, which is continued forwards and slightly upwards to be connected anteriorly with a small fibro-cartilaginous nodule lying to the outside of the olfactory chamber, and connected on its part with the premaxillary region.

The action of the first portion of the muscle is very evident, it being the true levator of the palatine arch, but also the dilatator of the operculum, as already mentioned. The second, third and fourth portions are diverted from their relations to the palatine arch, and act as assistants to the Add. mand., while as to the fifth portion it is difficult to say what its true functions

are, but apparently it has been specialized in some connection with the olfactory organ.

The *distribution of the Trigeminus* to these muscles takes place in the following way. It will be necessary to state, however, before entering on the description, that I have not studied in detail the peripheral nervous system, but only, as a rule, so far as it bore upon the innervation of the muscles. Van Wijhe has given an account[1] of the main branches of the various cranial nerves, but has not followed them out to their ultimate distribution in most instances, and Sagemehl[2] has also given a description of their mode of exit, and to a certain extent of their proximal portions. The descriptions given below will serve, therefore, in some cases to supplement his observations. The *R. buccalis*, *R. max. sup.* and *R. max. inf.* (Fig. 4) leave the cranium by the same foramen, separate ones existing for the *R. oph. sup.* and *R. oticus.* Before dividing into the *Rr. sup.*, and *inf. max.*, and *buccalis*, the trunk gives off a branch from its posterior surface, which shortly divides, one branch (*a*) passing to the anterior portion of the first division of the Lev. arc. pal., the other (*b*) passing behind that muscle, runs backwards, and probably supplies its posterior fibres as well as those of the second division, though I was not able to ascertain this positively. After the division of the main trunk from the anterior surface of the *R. max. inf.* a branch (*c*) is given off, which passes downwards and forwards to end in the fifth portion of Lev. arc. pal., giving off as it goes a branch to the fourth portion. A little below this, a branch (*d*) is given off to the Add. mand., and then the nerve (*inf. max.*) penetrates that muscle, giving off as it does so a cutaneous branch (*e*) which passes directly downward, immediately beneath the integument, and curves around the mandible.

On comparing these muscles with these of *Acipenser*, the only other Ganoid of whose muscles I find a description,[3] the large size of the Add. mand. is at once very noticeable; but here one

[1] Van Wijhe, *Ueber die Visceralskelet u. d. Nerven des Kopfes der Ganoiden und von Ceratodus.* Niederl. Archiv, Bd. V.

[2] Sagemehl, *Beitr. zur vergl. Anat. der Fische.* Th. I. Morph. Jahrb. Bd. IX, 1883.

[3] Vetter. *Unters. zur vergl. Anat. der Kiemen- und Kiefer-Musculatur der Fische.* II Theil. Jen. Zeit. Bd. XII, 1878. This paper treats of *Chimæra Acipenser*, and certain Teleostei.

must consider, from comparison with the Selachians[1] and *Chimæra*, and, on the other hand, with the Teleostei, that in this respect *Amia* more clearly resembles the original type than does *Acipenser*. The division of the muscle in *Amia* is, however, a commencement of the specialization into several parts seen in Teleostei, and which is carried to its greatest degree in the Cyprinoids. Into the muscle in these forms, however, probably other elements enter, as will be seen below.

With regard to the Lev. arc. pal., the relation of its second, third and fourth positions to the Add. mand. is at first somewhat confusing, and it is difficult to see what are the exact relationships of these muscles. To consider first the second and third portions. These from their origin are apparently equivalent to the muscle in *Acipenser*, termed by Vetter the Protractor hyomandibularis, but their insertion is somewhat different. There the Protr. hyomand. is inserted into the hyomandibular, here the muscles come into connection with the Add. mand. Both muscles are supplied by the Trigeminus, and by a branch from the yet undivided trunk of that nerve. The difference in the palatopterygoid arches in the two forms must also have had an effect in producing changes in the muscles. I am inclined, however, to consider the arrangement in *Acipenser* secondary, and to go further back in tracing the changes which have been undergone by this muscle. Vetter's homology of the Protr. hyoman. with the muscle he terms Lev. maxill. sup. in the Elasmobranchs, is evidently a quite correct one. Here we have a muscle whose origin is similar to that of the second and third portion of the Lev. arc. pal. of *Amia*, and which is inserted into the Palatopterygoid arch, and moreover comes into somewhat intimate relation with the Add. mand. An increase of these relations and the development of a metapterygoid would bring about exactly the arrangement one sees in *Amia*. A question arises, however, as to what becomes of these muscles in the Teleostei. I have already hinted above that the Add. mand. of *Amia* is not quite comparable to that of the Teleostei, but in the latter other parts become added to the originally simpler muscle.

[1] Vetter. *Unters. zur vergl. Anat. der Kiemen- und Kiefer-Musculatur der Fische.* I Th. Jen. Zeit. Bd. XIII, 1874. Treats of the Selachians.

I believe that these parts represent the second and third portion of the Lev. arc. pal. In *Amia* there is no slip from the Add. mand. passing to the maxilla, such as one sees in *Cyprinus*, *Barbus* and *Perca*,[1] and probably most other Teleosts, but a ligament extends to about the middle of the inner surface of that bone from the coronoid process of the mandible. When the mandible is depressed the coronoid is thrown forward, and by the slackening of the ligament thus produced the maxilla is allowed to move forwards, being again drawn back parallel to the axis of the body when the mandible is raised by the Add. mand. At its origin from the coronoid this ligament is in relation to the third portion of the Lev. arc. pal., and one can readily imagine the insertion of that muscle changing so as to unite with the ligament. At the same time, however, the muscle would require to lose its connection with the second portion of the levator and to unite with the fibres of the superficial portion of the Add. mand. The more superficial situation of this muscle when compared with the second portion, in addition to its insertion, would lead it to associate rather with the superficial than the deeper portion of the Add. mand. On the other hand, the second portion of the levator probably unites with the deeper portion of the Add. mand., its extension backward behind the first portion of the levator first disappearing, and then its connection with the cranium. Whether these two muscles have the destiny here assigned to them can only be determined by investigating intermediate conditions. These relations would, however, stand in confirmation of the statement already made in connection with the simplified Add. mand. of *Amiurus*, that this simplicity and that of *Esox* was in reality secondary, the more complicated arrangement of *Cyprinus*, for instance, being earlier.

The fourth portion of the Lev. arc. pal. is probably identical with the Levator anguli oris of *Chimæra*, and with the parts in the Selachians from which this has arisen. It is apparently wanting entirely in *Acipenser*. The fifth portion, however, may be compared with the anterior portion of the constrictor of *Acipenser*, although with considerable reticence on account of

[1] Descriptions of the musculature of these forms, and of *Esox*, will be found in Vetter's paper, Theil II, above referred to, and of *Amiurus* in my paper on that fish.

the uncertainty which exists in regard to the innervation of that muscle. It would, perhaps, be better to refer it to the same group of muscles as the fourth portion.

The first portion of the levator is very evidently comparable to the Lev. arc. pal. and Dilatator operculi of the Teleostei, and the relation of its posterior fibres to the operculum proves conclusively the supposition made by myself from the study of *Amiurus*, and by Vetter from that of other forms, *i. e.* that the dilatator of the operculum has been specialized secondarily from the Lev. arc. pal. To the homologies which Vetter draws between the Lev. arc. pal. of Teleosts and the Protractor hyomand. and Lev. max. sup. of *Acipenser* and the Selachians, respectively, I am compelled to take exception. Since these muscles have been shown to be equivalent to the 2d and 3d portions of the levator of *Amia*, we must have in the Lev. arc. pal. and in that of the Teleosts a muscle which is not represented in any forms below *Amia* which have as yet been studied, and which must be regarded as a specialization of a certain portion of the Add. mand. of such a form as *Acipenser* or a Selachian. For the same reason, then, the homology of the Add. mand. of the Elasmobranchs or of *Acipenser* with that of the Teleostei, or even with that of *Amia*, is not quite accurate, for it is equivalent to that of the latter *plus* the first portion of the Lev. arc. pal., and the muscle of the Teleosts is equivalent to the Add. mand. and Lev. max. sup. of the Selachians *minus* some fibres of the former which correspond to Lev. arc. pal. and Dil. op.

B.—The Facial Muscles.

The *Adductor hyomandibularis* forms a broad, but short muscle, extending from the auditory region of the skull, *i. e.* from the prootic and opisthotic regions, to the metapterygoid, hyomandibular and operculum, into the inner surfaces of which bones it is inserted, and, accordingly, this muscle might with equal propriety be termed the Adductor arcûs palatini, since it is equivalent both to that muscle and to the Add. hyomandibularis, and also to the Add. operculi of Teleosteans. It is also evidently equivalent to the Retractor hyomand. and to the opercularis of *Acipenser*. It is to be noticed, however, that this latter muscle is of much greater extent in *Acipenser* than in

Amia, due, of course, to the rudimentary condition of the operculum in the former, and it is also to be noted that in the Teleostei those fibres which are inserted into the upper border of the bone become separated from those inserted into the inner surface as a distinct muscle, the Lev. operc., so that the muscle of *Amia* is equivalent to this as well as to the other three Teleostean muscles already mentioned.

The facial nerve (Fig. 5) after issuing from the skull passes downwards and backwards to penetrate the hyomandibular as in the Teleostei, but in its course gives off only one branch, the *R. opercularis* (ROp), as it may be termed, which supplies the muscle just considered. In Teleostei, two branches or more are given off, the *R. ad. musc. add. arc. pal.* and the *R. operc.*, the former of which, as its name indicates, passes forward to the Add. arc. pal., and the latter backwards to the Add. operc., while, according to Vetter, other branches are given off for the Add. hyomand. In Teleosts, however, the facial nerve passes either between the Add. arc. pal. and the Add. hyomand., or even traverses the latter, so that a considerable portion of muscle lies in front of the nerve, for which a special branch (or branches) has become specialized; but in *Amia* the entire muscle, with the exception of a very small portion anteriorly which overlaps, lies behind the nerve. Functionally, then, this *R. opercularis* must be considered equivalent to all the branches given off in higher form from the facial in its course from the cranium to the hyomandibular canal.

The *Intermandibularis* (Fig. 2, IM) is reduced to a very small portion indeed, lying close to the symphysis of the mandible, its fibres extending transversely across between the two rami. It corresponds, in the absence of a median aponeurosis, exactly with the muscle of that name in the Teleostei, and there are no reasons for disputing its homology with the mylohyoideus (Cs_6) of *Acipenser*. It should, probably, as the sequel will show, have been described with the trigeminal muscles, but has been placed in the facial group in order that its innervation might be discussed along with that of the geniohyoid.

The *Geniohyoid* (Figs. 1 and 2, GH) arises posteriorly from the outer surface of the ceratohyal, as far back as the level of the eighth branchiostegal ray. The outer fibres run almost directly

forwards, parallel to the ramus of the mandible, and are inserted anteriorly into the integument forming the floor of the mouth; the innermost fibres, however, curve inwards in front, and are inserted into a median aponeurosis common to it and to its fellow of the opposite side. In addition to this portion there is anteriorly a portion (Fig. 2, GH²) which might almost be described as a reduplication of the inner fibres of the muscle upon themselves. This portion, which lies superficially to the rest of the muscle, and which is covered by the large integumental ossification lying behind the symphysis of the mandible (Fig. 1, Men), is a continuation forwards of the inner fibre of the muscles into a superficial layer, which is partly separated from the lower fibres, and which, extending forwards and slightly outwards, is inserted into the ramus of the mandible behind the attachment of the intermandibularis. The superficial portions of either side are in rather intimate relation. The resemblance to the Genio-hyoid of *Esox* is very marked.

The *Hyohyoideus* (Figs. 1 and 2, HH) arises on either side from the ceratohyal and anterior branchiostegal rays below the origin of the geniohyoid. Each muscle passes forwards and inwards. The outermost fibres converge to a strong tendon inserted into the cartilaginous part of the hypohyal, while the inner ones pass across the middle line, those of the right side overlapping, *i. e.* passing dorsally to, those of the left, and are lost in the fascia covering the muscle and the hypohyals of the opposite side. Behind the point of origin given above, fibres of the muscle can, however, be seen passing backwards over the proximal extremities of the anterior branchiostegal rays to the lower edge of the ceratohyals. The muscle accordingly, like the geniohyoid, bears much resemblance to that of *Esox*, while it differs somewhat from that seen in *Perca* and that of *Amiurus*. In the latter more especially the muscle is simpler, but this simplicity must be considered a secondary acquisition, and not a representation of the earlier condition. A point in which the Teleostei differ from *Amia* is in the relation of the muscle to the branchiostegal rays. In the Teleosts the muscle extends back as far as the operculum or suboperculum, either forming a continuous sheet on the inner surface of the rays (*Esox*), or else passing from the lower border of one ray to the upper border

of the next succeeding (*Amiurus*). In *Amia* we have a faint trace of a commencing differentiation of this portion of the muscle, seen in muscular bands, consisting of few fibres, which run in the branchiostegal membrane, extending out from the lower border of the ceratohyal, and quite distinct from the main portion of the muscle. These bands do not show any definite relation to the number of the rays, but one band may extend over a length occupied by the articulations of several of them, evidently showing that the continuous arrangement seen in *Esox* must be considered the earlier one.

I regret that I have not been able to determine accurately the innervation of certain of these muscles, but the probable innervation can be deduced from the arrangement of the nerves. It will be necessary to describe the arrangement of the trigeminal and facial nerves. The trigeminal (Fig. 4), after perforating the Add. mand. in the manner above described, runs along in the substance of that muscle until the termination of its insertion comparatively far forwards into Meckel's cartilage, giving off during its course one branch upwards and another downwards. The membrane bones of the mandible require to be cut away to expose the muscle and nerve in this region. Opposite the anterior end of the insertion of the muscle, where the nerve leaves it, a branch (f) is given off, which, passing through a foramen in the mandible, is distributed to the integument and to the anterior superficial portion of the geniohyoid muscle. The *Inf. max. trig.* is then continued forward in the substance of the dentary, the *R. mand. fac. ext.* (Fac) uniting with it. I was unable to trace the united nerve quite to its termination, but followed it to within a short distance of the symphysis.

The facial (Fig. 5) after leaving the hyomandibular canal, divides into two branches, the *R. mandibularis* (RM), and the *R. hyoideus* (RHy). I could detect no twigs passing to the Add. mand. as Vetter describes in *Esox*. The *R. mand.* soon divides into two branches, the external (RME) and internal (RMI); the latter I did not succeed in following any further than Van Wijhe has done. The *R. mand. ext.* however passes down behind the ligament uniting the interoperculum with the mandible, and then curves forwards round its lower border and enters the mandible more anteriorly, running between the inner

lamella of the dentary and Meckel's cartilage, and finally in a canal in the dentary, eventually uniting with the *R. inf. max. trig.* as above described. The *R. hyoideus* after its origin passes backwards and downwards across the hyomandibular, below the preoperculum, and then runs in the branchiostegal membrane between the bases of the rays and the ceratohyal, passing into the hyohyoideus, which it supplies.

The innervation of the hyohyoideus corresponds exactly with what has been observed for various Teleostei. I believe that the hyoid branch of the facial, in addition to supplying this muscle, also sends branches to the posterior portion of the geniohyoid, so that this muscle receives branches from both the trigeminal and the facial. Vetter describes the anterior portion of the muscle as being innervated by a branch from the conjoined trigeminus and facial, and behind by a branch from the *R. hyoideus facialis.* When we consider that the union of the two nerves is delayed in *Amia* until they have passed comparatively far forwards, we will have an explanation of the trigeminus only contributing to the anterior innervation. The branch which that nerve gives off before the union is apparently quite equivalent in its point of exit and distribution to the branch from the conjoined nerves in the Teleosts, and perhaps even in these forms the fibres which pass into the conjoined trunk from the facial do not take part in the formation of the branch to the muscle. That, however, can only be determined by observation of the effect produced by stimulation of the facial branch before its union with the trigeminus, and indeed this physiological method might with much advantage be put in practice for determining the innervation of muscles when fresh specimens are available. The intermandibularis may perhaps be innervated by a branch given off quite close to the symphysis from the conjoined nerves, or else by a twig from the branch of the trigeminus which supplies the anterior portion of the geniohyoid. I prefer the latter idea, for although the former one would make the innervation occur from a branch in which both the trigeminus and the facial were united, yet in no fish, not even in Elasmobranchs, do I find any nerve described as issuing anteriorly to that which supplies the anterior part of the geniohyoid, and in the Teleosts Vetter has described a branch

from this nerve as innervating the intermandibularis. If this be the correct innervation, then the intermand. is really a trigeminal muscle, corresponding to the anterior portion of the constrictor of Elasmobranchs which is supplied by the trigeminus. Its probable relation to the mylohyoid (Cs_6) of *Acipenser* is also much in favor of this view, that muscle being innervated by the trigeminus. I am therefore, in view of these considerations, strongly inclined to withdraw the observations I made on the development and equivalency of the intermand. in my paper on *Amiurus*, and to apply them to a certain extent to the geniohyoid; for this muscle from its innervation by the trigeminus, and also probably by the facial, in addition to its very evident relation to the hyohyoideus, which is essentially a hyoidean muscle, evidently corresponds to or is made up of a portion of the anterior portion of the constrictor supplied by the trigeminus, and the posterior portion supplied by the facial.

C.—Muscles supplied by the Glossopharyngeus and Vagus.

1. *Ventral Muscles.*

The *Interarcuales ventrales* (Fig. 3, Iav_5), to adopt the name applied by Vetter to similar muscles in *Acipenser*, present a slightly more complicated arrangement than in that form, being for the most part double, *i. e.* two muscles to each arch. The muscle of the first arch ($Iav_1{}^a$) arises from the posterior surface of the hypohyal,[1] and passing backwards and outwards is inserted into the lower extremity of the hypobranchial of the first arch, the second portion ($Iav_1{}^b$), arising from the posterior border of the hypobranchial of its arch, being inserted into the corresponding ceratobranchial. The interarcuales of the second arch (Iav_2) arise respectively from the posterior extremity of the anterior cartilaginous portion of the first basibranchial and from the posterior border of the HBr_2, and both portions, passing outwards, are inserted into the lower extremity of CBr_2. So with the third arch (Iav_3), one portion arising from the posterior end of the osseous BBr_1 (which probably should more properly be termed BBr_2 as designated in the figure), and the other from

[1] In speaking of the bones of the branchial and hyoid arches I have employed the terms employed by van Wijhe in the paper already referred to.

the posterior edge of the HBr_3, both being inserted into the extremity of the CBr_3; and so also with the fourth arch (Iav_4), (van Wijhe's posterior cartilaginous BBr_1, being probably more correctly described as the BBr_3).. With the fifth arch, however, there is a difference, its interarcual (Iav_5) having shifted its position very remarkably, so that it arises from the anterior extremity of what I have called the BBr_3, and passes thence backwards, crossing the interarcuales of the fourth arch and part of the anterior transversus ventralis anterior, and, becoming tendinous, is inserted into the anterior border of the single bone of the fifth arch, which probably represents the ceratobranchial.

The innervation of these muscles presents little of any great importance, the first being supplied by branches from the glossopharyngeal, and the others from the vagus of their respective arches. The fifth arch, however, presents some peculiarities, and apparently the vagus trunk to the third arch supplies the muscle which passes to it. On some grounds one would have expected for it an innervation from the vagus branch to the fifth arch, but on the other hand its relation to the third arch would render probable what does occur.

In *Acipenser* these muscles are all quite simple, so that *Amia* presents an increased specialization, which is carried still farther in the Teleosts, one of the portions of each muscle of *Amia* becoming probably converted into the Obliquus ventralis seen in these higher forms. The true interarcual of the fifth arch, as will be seen below, probably becomes converted into the transversus ventr. posterior, the arch being thus left destitute of an interarcual, which, however, is supplied it by the third arch, the muscles of the fourth arch being specialized in another direction, so that the muscle has in reality passed backwards, instead of going forwards as one might at first suppose.

The *Transversi ventrales* are two in number, as in most higher forms. The anterior muscle (Fig. 3, Tva) arises from the fourth hypobr. principally, its posterior fibres being inclined backwards so as to arise from the fifth arch, the muscle being thus broader behind than in front. It passes across the middle line, and is inserted into the corresponding parts of the opposite side. The posterior muscle (Tvp), on the other hand, is entirely confined

to the fifth arch, arising from the greater part of the posterior edge and surface of the single bone representing that arch, and passing across to the arch of the opposite side, an indication of a median raphe being present.

The Trans. vent. ant. is supplied by the trunks of the vagus for the fourth arch of either side; the posterior, by those of the fifth arch.

There can be no doubt that these muscles consisted originally of two distinct muscles, which have fused together so as to form an unbroken sheet across the middle line. Their innervation by the trunks of both sides shows this. In *Acipenser* there is no Transv. ant., and the post. is in that form very plainly the interarcualis of the fifth arch. So, too, it must be regarded here, it having the position occupied by the interarcuales of the preceding arches. The Transv. ant. seems to be, however, a specialization of the interarcuales of the fourth arch, both from its origin and innervation.

2. *Dorsal muscles.*

The *Levatores arcuum branchialium ext.* arise as two muscles immediately internal to the origin of the Add. hyomand. The anterior one is the smaller, and passing outwards and slightly backwards, is inserted near the distal extremity of the epibranchial of the first arch. The posterior muscle lies immediately behind this and has a similar direction, but soon splits up into three divisions, passing respectively to the epibr. of the second and third arches, and to the single bone (pharyngobranchial ?) of the upper moiety of the fourth arch, a few fibres from this being continued towards the degenerated fifth arch.

The anterior muscle is supplied by a branch from the *Truncus branch. glossopharyngei*, and to each of the three divisions of the posterior muscle a branch passes from the corresponding *Tr. branch. vagi.* In *Acipenser* the Lev. branch. are more numerous, there being altogether five, according to Vetter, and in the Teleosts they are also more numerous, seven altogether being present in *Amiurus*, three of which, however, are to be referred to the group presently to be described. It is also to be noticed that in *Acipenser* many of the muscles arise from the terminal pieces of the arches, whereas in *Amia* and the Teleosts they all arise from the skull. It

would seem as if these muscles were analogous to the interarc. vent., but have subsequently lost their relation to the arches as far as their origins are concerned, and have been transferred to the skull.

The *Lev. arc. branch. int.* consists of two muscles, the anterior of which is the smaller, and lies on the inner side of the anterior Lev. arc. branch. ext. It is, however, directed downwards and inwards, and is inserted into the pharyngobranchial of the second arch, being supplied by a twig from the *Trunc. branch. glosso-pharyngei.* The posterior muscle arises close beside the anterior, and is directed somewhat backwards as well as downwards and inwards, passing between the first and second branchial trunks of the vagus, and is inserted into the upper surface of the Os pharyngeum sup. formed on the pharyngobranchials of the third and fourth arches. It is apparently supplied by a twig from the R. branch. vagi II. These muscles are evidently quite comparable to those described under the same name by Vetter in *Perca*, only differing slightly from those of *Cyprinus*. The innervation is apparently different, but I cannot speak with absolute certainty as to the correctness of that given above for *Amia.*

The *Transversus dorsalis* is simply a continuation forwards of the constrictor muscles which surround the pharynx, and accordingly is probably innervated by a branch for the pharyngeal nerves of the vagus.

D.—Muscles supplied by the first spinal nerve.

The *hyopectoralis* (sterno-hyoid) arises from the upper surface of the ventral portion of both clavicles, the portions of opposite sides being united, but indicated by a longitudinal groove along the middle line of the muscle, and by a median aponeurosis. Anteriorly the two portions separate, each passing into a rounded tendon which is inserted into the posterior surface of the hypohyal of the corresponding side. To the sides of the muscle of each side are attached the two *flagella*, the anterior one being bound down to the integument, while the posterior one is movable by the contraction of the muscle. Between the two portions of either side is a vertical median aponeurosis, into which fibres from both sides are inserted. From this aponeurosis, which is most marked

behind and towards the upper (dorsal) surface, a tendon passes forwards and upwards, dividing into two portions, each of which passes to a hypobranchial of the second arch.

This muscle is supplied by a branch from the first spinal nerve.[1] Leaving the spinal column in front of a partially aborted vertebrae, the first spinal nerve passes downwards, uniting with a small nerve which passes out of the ex-occipital behind the vagus foramen, and which arises by a single (ventral) root. The conjoined branch then passes downwards in front of the pectoral arch, immediately below the membrane which extends from the fifth arch to the pectoral girdle. On nearing the hyopectoralis it divides, one branch passing into the posterior portion of the muscle; the other, continuing on the course of the main branch, runs forwards in the substance of the muscle.

There can be no doubt as to the comparability of this muscle with the coracoarcualis of *Acipenser*, which again is comparable to the coracoarcualis and the coracobranchiales and coracohyoideus of the Elasmobranchs, the mode of the transition being as indicated by Vetter. The slips to the branchial arches forming the coracobranchiales of the Elasmobranchs gradually become non-muscular and represented only by tendons as seen in *Acipenser*, going to the hyoid and three anterior branchial arches. The reduction then affects these, which become reduced to a single pair, those of the second arch, which are in relation to the muscle immediately to be described, and, finally, this pair also disappears, and we have the arrangement characteristic of the Teleostei. With the disappearance of the coracobranchiales the relative stoutness of the coraco-arcualis, of which the coracohyoideus is merely the anterior portion, increases.

The *Branchio-mandibularis* (Figs. 2 and 3, BrM) arises by two heads from the mandible on either side of the symphysis above, *i. e.* dorsal to, the point of insertion of the intermandibularis. The two portions unite very soon, and pass backwards dorsal to the median aponeurosis of the geniohyoideus, and ventral to the symphysis of the hypohyals, becoming at this point somewhat tendinous. It then passes dorsally (upwards) between

[1] This nerve is referred to in the second portion of this paper as the second post-occipital nerve, but there is no doubt but that it is a true spinal nerve.

the two tendons of the hyopectoralis. It then again divides into two portions, each of which passes a little outwards, to be inserted respectively into two tendons which pass from the median aponeurosis of the hyopectoralis to the second branchial arches. This muscle will evidently, the mandible being fixed, assist in depressing the branchial arches; or if the arches are fixed, will assist in depressing the mandible.

The innervation of this muscle, I regret to say, I was unable to trace out. I feel quite confident, however, that it is supplied by the first spinal nerve. The anterior branch of that nerve, as described above, enters the substance of hyopectoralis and passes forwards. I was able to trace it for a considerable distance during which it passed in towards the middle line of the muscle, and I have no doubt that still more anteriorly it passed over to the branchio-mandibularis. The relationship of this muscle to the hyopectoralis, which is even more apparent in lower forms, would lead one to expect the same innervation. Vetter, too, was unable to trace its innervation in *Acipenser*, and Stannius,[1] according to the same author, does not mention it. The coraco-mandibularis of the Elasmobranchs is supplied by the anterior spinal nerves, and this muscle is quite comparable in its relations to the branchio-mandibularis.

In the Teleosts we find no trace of this muscle, and *Amia* is probably the last piscine form which presents it, as one ascends the scale. It is found, however, in the lower forms, as in *Acipenser*, where it is somewhat better developed, and differs from that of *Amia* in allowing the tendon from the hyopectoralis to pass through it, and in being inserted directly into the hypobranchials of the *third* arch. In Elasmobranchs it is clearly an anterior continuation of the coraco-arcualis, named by Vetter the coraco-mandibularis, and is in these forms a large stout muscle. It must be noticed in thisc onnection, that in all forms below the Teleosts there is a continuous sheet of longitudinal muscles extending from the mandible to the trunk, and belonging to the system of the trunk musculature. In the Teleosts, however, these longitudinal muscles do not extend any farther forwards than the

[1] Stannius. *Das peripherische Nervensystem der Fische.* Rostock, 1849. This paper I was not able to consult.

hyoid. In my paper on the musculature of *Amiurus* I suggested that in primordial forms there was a continuous sheet of this kind. If this be so, it has disappeared entirely in the region occupied by the mandibular, hyoid and branchial arches, and in its stead the ventral musculature of the trunk has grown forwards. This is possible. The hyobranchialis of *Amiurus* must not, at any rate, be considered as representing the ventral longitudinal musculature of the hyoid and branchial arches, for the longitudinal direction of its fibres is very evidently, from comparison with such forms as *Amia* and *Acipenser*, a secondary affair.

The *pharyngo-claviculares ext. and int.* (Fig. 3, Phc 1 and 2) arise close together, in fact as one muscle, from the clavicula on the outer side of the hyopectoralis. As they pass inwards and forwards the two muscles separate, the externus passing to the single bone representing the fifth arch, being inserted at about the inner third of the bone; the internus passing more inwards, is inserted into the same bone towards its inner end and partly into the basibranchial, which succeeds the fifth arch (BBr 5), termed the basibr. iii by van Wijhe, but which should perhaps be called the basibr. v.

These muscles are innervated by branches from the first spinal nerve, just before the main branch as above described passes into the substance of the hyopectoralis. These muscles resemble very closely those of Teleosts, with which they are homologous. In *Acipenser* they are apparently wanting, but in Elasmobranchs Vetter describes a muscle, the fifth coraco-branchialis of *Acanthias*, and the seventh coraco-branchialis of *Heptanchus*, which is very similar in its relations, and the pharyngo-clav. of higher forms may very probably be a specialization of it.

Summary.

1. The protractor hyomandibularis of *Acipenser* and Lev. max. sup. of the Elasmobranchs are represented by a series of muscles, which may be considered parts of the Lev. arc. pal. system, but which do not correspond with the Lev. arc. pal. of Teleosts, becoming in these forms reduced, and uniting with the Add. mand.

2. The Lev. arc. pal. of the Teleosts is seen, from what occurs in *Amia*, to be a new structure not represented in lower forms, and separated off from the Add. mand.

3. There is no distinct Dil. operc., but the posterior fibres of the Lev. arc. pal. proper have assumed an oblique direction and perform its functions.

4. The intermandibularis and anterior portion of the geniohyoid are supplied by a branch from the fifth nerve before the union with it of the seventh.

5. The coraco-branchiales of the Elasmobranchs, which are represented in *Acipenser* by tendinous slips to the branchial arches, are reduced in *Amia* to a single pair of tendons passing to the second arch.

6. A branchio-mandibularis is present.

Part II.

THE RELATIONSHIPS OF THE POST-OCCIPITAL AND HYPOGLOSSAL NERVES.

The basioccipital of *Amia* is produced backwards some distance behind the level of the plane of the posterior surface of the cranium, and on the posterior portion of this prolongation are to be seen the rudiments of two neural arches. Behind the opening for the vagus there passes out through the exoccipital a fine nerve, which arises by a single root; and in front of each neural arch are two foramina, which give passage to a dorsal and ventral root respectively of a nerve. The anterior fine nerve unites, after passing down a short distance, with the second, the two then passing down on the membrane at the back of the pharynx; the conjoined trunk dividing, one branch passes to the posterior portion of the hyopectoralis, while the other is continued forwards, supplying the pharyngo-claviculares and eventually probably the branchio-mandibularis. The third nerve passes downwards and backwards below the pectoral arch, and unites with what may for convenience be called the first spinal nerve, which is distributed, with four other nerves, to the pectoral fin muscles.

The partial fusion of the vertebrae corresponding to these nerves with the skull in *Amia* has led Sagemehl to formulate

a theory,[1] as follows: "Der Umstand dass dem Occipitale basilare diskrete obere Bogen aufsitzen, zwischen welchen nach dem Typus der Spinalnerven gebaute Nerven austreten, ist für die Beurtheilung der Schädel der höheren Fische von fundamentaler Bedeutung und lässt keine andere Deutung zu, als dass mit dem ursprünglichen Primordialcranium, das wir bei Selachiern am vollkommensten ausgebildet finden, noch mehrere Wirbel sammt den zu ihnen gehörigen Nerven verschmolzen sind." Such a theory can only be accepted when more facts are brought to bear favorably upon it, and such facts can only be obtained by a study of the various parts in relation to these post-occipital nerves and vertebrae, and from the embryological history of the basioccipital region, etc., in the higher fishes. This last part of the subject I have been unfortunately unable so far to investigate, but a study of adult forms leads me to doubt the absolute correctness of the theory. To determine its accuracy it is necessary first of all to see whether in the Teleosts any trace of these vertebrae and their parts which are supposed to be taken up into the skull is to be found. From a study of the crania of the Characinidæ[2] Sagemehl believes the parts to be represented by the auditory bones in part, the nerves having for the most part disappeared, or more in detail, the first nerve of *Amia* (passing out of the exoccipital) has disappeared; the second is represented by a nerve passing out of the exoccipital in the Characinidæ behind the vagus, the osseous bar fused with the exoccipital being equivalent to its neural arch; while the third nerve has disappeared, but its arch is represented by the claustrum. One cannot avoid noticing the enormous changes undergone in the parts in these higher forms, nor is it easy to believe that the identifications are correct in the absence of intermediate conditions. Can such intermediate conditions be found? Sagemehl states that discrete occipital arches are to be found in *Esox*, Salmonidæ, and Clupeidæ, and if this be the case we certainly have structures which appear to be intermediate, and in fact there is no reason why the rudimentary arches present in

[1] Sagemehl. *Beitr. zur vergl. Anat. der Fische.* I. *Das Cranium von Amia Calva L.* Morph. Jahrb. Bd. IX, 1883.

[2] Sagemehl. *Beitr. zur vergl. Anat. der Fische.* Th. III. *Das Cranium der Characiniden, etc.* Morph. Jahrb. Bd X, Heft 1, 1884.

Amia should not persist in the lower Teleosts. The identification of the claustrum with one of these arches is very tempting, and not improbable, though the interpretation given by Ramsay Wright,[1] that it represents an intercalar ossicle similar to what Götte has described as occurring in *Esox*, is perhaps to be preferred, in view of the probable presence of other intercalar arches in *Amiurus*. But, even if Sagemehl's views on these subjects are correct, we see the occipital neural arches in such highly organized Teleosts as *Amiurus*, the Characinidæ, etc., remaining free, and *not* fusing with the skull.

More information is, however, to be obtained from the nerves —structures which usually retain their original relations longer than the bones. It was seen above that the distribution of the two anterior post-occipital nerves of *Amia* was to the hyopectoral, pharyngo-claviculares and branchio-mandibularis. In all Teleosts hitherto examined, the hyopectoral and pharnyngo-claviculares are innervated by the first spinal nerve, while the last named muscle is unrepresented. From this, one would conclude that the first and second post-occipital nerves had either become converted into the first spinal nerve, or had fused with the third post-occipital to form the first spinal. This third post-occipital was seen to unite with the first true spinal to pass to the pectoral fin musculature, and the first spinal nerve of the Teleosts also takes part in the formation of the brachial plexus. It is evident, therefore, that the first spinal nerve of the Teleosts has usurped the functions of the post-occipital nerves of *Amia* in addition to retaining its own, or, in other words, the post-occipital nerves have in the Teleosts passed backwards and become incorporated with the first spinal. Whether the first nerve passes backwards with the other two is doubtful, in fact improbable, and I am inclined to agree with Sagemehl with regard to it that it has become absorbed. It is different from the two posterior nerves, both in its origin from a single ventral root, and in passing out through the exoccipital, two points in which it resembles vagus roots; and perhaps it is not to be compared to a spinal nerve (using the term in a restricted sense), but to one or more of the

[1] Ramsay Wright. *On the Nervous System and Sense-organs of Amiurus.* Proc. of Can. Inst., Toronto, Vol. II, 1884.

independent vagus branches shown by Gegenbaur[1] to pass out from the cranium of Elasmobranchs behind the foramen for the vagus proper. An interesting confirmation of the supposed fate of the two posterior nerves, and of the degeneration of the first nerve, is to be found in what occurs in *Gadus*, and no doubt in other forms also. In regard to the first spinal nerve of *G. Callarias* Stannius[2] makes the following statement: "Er entspringt mit einer starken hinteren und zwei starken vorderen Wurzeln. Sämmtliche Wurzeln verlassen den Canalis spinalis durch ein gemeinsames Foramen intervertebrale, das an der Grenze des Hinterhauptsbeines und des Processus spinosus des ersten Rückenwirbels liegt. Unmittelbar nach dem Austreten trennt sich die hintere Wurzel in zwei Bündel, von welchen jedes ein eigenes Ganglion besitzt. Indem die vorderen Wurzeln an die beiden Bündel der ursprünglich einfachen hinteren Wurzel sich anlegen, entstehen zwei Nervenstämme." There is every reason to suppose that these "two strong anterior roots" are representatives of the second and third post-occipital nerves of *Amia*, and it is seen that there is no trace of the first nerve, which is not a true post-occipital nerve.

In some Teleosts a nerve passes out apparently through the exoccipital behind the vagus, and is distributed to the same part as the post-occipital nerves of *Amia*. This nerve, commonly identified as the hypoglossus, is simply the first spinal nerve which has passed forwards so as to make its exit through what may be termed the arch of the preceding vertebra, exactly as the succeeding nerves may do. This is shown by the fact that where it occurs there is no nerve passing out immediately in front of the first vertebra, or through its arch. In *Amiurus* the nerve penetrates the exoccipital twice, once in passing into the interior of the cranium, and again in passing out; and in many forms, *e. g. Gadus*, as seen from the quotation given above, the nerve passes out, not through the exoccipital at all, but in front of the arch of its vertebra. There is accordingly a gradual passage from the normal arrangement, where the nerve passes out in

[1] Gegenbaur. *Untersuch. zur vergl. Anat. der Wirbelthiere.* Theil III. Leipzig, 1872.

[2] Stannius. *Ueber das periph. Nervensystem des Dorsch, Gadus Callarias.* Müller's Archiv. Jahrg. 1842.

front of or through the arch of its vertebra, to the secondary, where the nerve passes up into the skull through the foramen magnum, and makes its exit through a post-vagal foramen in the exoccipital.

Although the nerves have passed backwards there are no mechanical difficulties in the way of the fusion of the centra of the corresponding vertebrae with the skull, and I am inclined to believe this to have occurred. In the youngest specimen I have been able to examine I have found no trace of such a fusion, but nothing can be deduced from that, since in *Acipenser*,[1] which also possesses post-occipital nerves, the notochord anteriorly is surrounded by a sheath of true cartilage at an early stage, which sheath is continuous in front with the parachordal cartilages. Now the neural arches make their appearance before the centra are indicated, and the basioccipital and the anterior vertebral centra might ossify as a whole without any individual centra being indicated, so that the neural arches would come to stand over the apparently simple basioccipital. The elongation of this bone behind the plane of the posterior surface of the cranium, seen in *Amia* and *Acipenser*, and to a much more noticeable extent in *Lepidosteus*, appears to indicate that there has been such a fusion of more or fewer vertebral centra with the cranium.[2]

It will be well to turn to the consideration of the arrangement of the corresponding parts in other forms. First, it will be necessary to examine *Petromyzon*. Wiedersheim, in his description of the nervous system of this form,[3] described the vagus as representing seven roots in *Ammocetes*, while the hypoglossal corresponded to eight roots, *i. e.* four dorsal

[1] Parker. *On the Structure and Development of the Skull in Sturgeons* (*Acipenser ruthenus and A. sturio.*) Phil. Trans. Vol. 173, 1882.

[2] Since writing the above, Professor Ramsay Wright informs me, in reply to an inquiry, that from the study of a series of sections of young specimens of the last-named form, he makes out that "On the plane of the Chorda there are two or three horizontal sections, which show a distinct limit between the cranial cartilage and the cartilage investing the anterior part of the vertebral notochord," in other sections they are continuous. From this there can be no doubt as to the fusion in *Lepidosteus*, and the probabilities are exceedingly strong for a similar arrangement in *Amia*.

[3] Wiedersheim. *Das Gehirn von Ammocetes und Petromyzon Planeri, etc.* Jen. Zeit. Bd. XIV, 1880.

and four ventral. His identifications, however, called forth criticism from Schneider,[1] and more recently from Ahlborn,[2] the latter author showing that the vagus really includes the anterior dorsal root of Wiedersheim's hypoglossus, while the posterior root belongs to the second spinal nerve, and the two roots anterior to this form the first spinal nerve, leaving only three motor and one sensory nerve to be accounted for. The hypoglossus of *Petromyzon* is formed of three motor roots, the sensory root probably disappearing, so that there can be but little doubt but that the hypoglossus really corresponds to three nerves of *Ammocetes.* The nerve after the three roots have united passes downwards and forwards to supply the muscles of the tongue. I have not been able to study the musculature of *Petromyzon,* nor to consult the work of P. Fürbringer on the subject, but from analogy I have no doubt that the distribution is similar to that of the post-occipital nerves of *Amia.* It has been seen that in the Teleosts the first spinal nerve is composed of three portions, and so it is in *Petromyzon.* In its point of exit, too, this so-called hypoglossus corresponds to the first spinal of Teleostei. Throughout the series of rudimentary arches the dorsal roots of the nerves pass out between the arches, the ventral roots through them. The most anterior arch, however, differs, both the dorsal and ventral roots passing through it, and immediately in front of it lies the hypoglossus. What has happened has probably been that the arch corresponding to the hypoglossus has fused with that for the spinal nerve, and the former nerve has passed slightly forwards to lie in the position normal to higher forms. Accordingly then the hypoglossal is really the first spinal nerve, comparable to the first spinal and two post-occipital nerves of *Amia* and the first spinal of the Teleostei.

As regards the Dipnoi there is greater difficulty. Wiedersheim in his description of *Lepidosiren*[3] describes as the hypoglossal, two nerves which leave the skull through the supra-occipital

[1] Schneider. *Ueber die Nerven von Amphioxus, Ammocetes u. Petromyzon.* Zool. Anz. 1880.

[2] Ahlborn. *Ueber den Ursprung u. Austritt der Hirnnerven von Petromyzon.* Zeit. f. Wiss. Zool. Bd. XL, 1884.

[3] Wiedersheim. *Das Skelet und Nervensystem von Lepidosiren annectens* (*Protopterus ang.*). Jen. Zeit. Bd. XIV, 1880.

(which descends to the exoccipital region), both in front of the two partially aborted post-occipital arches. These nerves have a very similar relation to the post-occipital nerves in *Amia*, both uniting to form a stem with which a branch from the vagus on the one side and the first spinal on the other side unite, the composite stem then passing to form the brachial plexus. Beauregard,[1] whose descriptions in all points can not be depended upon with much safety, describes a very similar distribution for *Ceratodus*, the axillary plexus being made up of branches from the vagus, the two hypoglossal stems and two spinal nerves. Humphry[2] speaks of a "coraco-hyoid" nerve in *Lepidosiren*, which passes down behind the branchiae, and is distributed to the ventral muscle passing from the coracoid to the hyoid, which nerve no doubt is a part from the composite stem containing the hypoglossus, the muscle being evidently the hyopectoralis. There can be little doubt but that these two hypoglossal nerves are comparable with the two posterior post-occipitals of *Amia*, but the position of the second also in front of the rudimentary arches is peculiar. Further observations may show that the original position of the second nerve was between the two arches. In neither *Petromyzon* nor the Dipnoi, however, can the entire segment be said to have passed up into the skull, for even in the case of the former, where the concentration is most complete, the dorsal portions of the three myotomes remain perfectly distinct.

When one turns to the Amphibia, one finds many interesting facts. The Urodela being, as all observers are agreed, the more primitive group of the two into which the Amphibia are usually divided, it will be well to turn first to them. The first nerve passes out through the arch of the first vertebra, and is distributed mainly to the thoracico-hyoideus (hyopectoralis) and the maxillo-hyoideus.[3] The second nerve passes out between the first and second vertebrae, sends twigs to the thoracico-hyoideus and

[1] Beauregard. *Encephale et nerfs craniens du Ceratodus Forsteri.* Journ. de l'Anat. et de la Phys. Tome XVI, 1881.

[2] Humphry. *The muscles of Lepidosiren annectens with the cranial nerves.* Journ. of Anat and Phys. Vol. XI, 1872.

[3] These are the terms employed by Hoffman in Bronn's Klassen und Ordnungen des Thierreichs. Amphibia. Leipzig, 1878.

maxillo-hyoideus, and then divides into three portions, two of which are distributed to muscles of the shoulder-girdle, while the third unites with the third nerve, and goes with it to the brachial plexus. There can be little doubt but that the thoracico-hyoideus is equivalent to the hyopectoralis (sterno-hyoid) of fish, but the question arises as to what is the homologue of the thoracico-hyoideus. This muscle, which has also been described as the geniohyoid, is exposed on removal of the intermaxillares anterior and posterior, and consists of two narrow portions stretching from behind forwards to be inserted into the posterior surface of the mandible near the symphysis; behind it arises from the ventral surface of the basi-branchial which follows the body of the hyoid, and in *Proteus* is imbedded between the two sterno-hyoidei. From the relations of this muscle, not to the hyoid proper, but to the branchial arches and to the sterno-hyoid, it cannot be identified with the geniohyoid of fishes, but seems to be equivalent to the branchio-mandibularis of *Acipenser* and *Amia*. The equivalent of the geniohyoid of fishes is to be looked for in the mylohyoid (intermaxillaris anterior), the hyohyal being represented in part by the stylohyoid (intermaxillaris posterior), and also in part by the ceratohyoidei.

In the Urodela accordingly, the first spinal nerve is distributed to the same parts as the second post-occipital of *Amia*. The second nerve does not, however, pass entirely backward to fuse with the third and go to the brachial plexus, but also sends twigs to aid the first nerve, supplies some muscles connected with the pectoral arch on its own account, only a portion of it passing to unite with the third nerve. For several reasons, founded among other points on the embryonic history and the larval development, I am inclined to trace back the origin of the Amphibia to forms much more ancient than any of our existing Ganoids, so that for an extremely long time the Fish and the Amphibia have been undergoing a specialization in quite different directions, and one must not be surprised to find the Amphibia retaining certain structures in a more ancestral condition than do the Teleosts or even the modern Ganoids. The retention of the branchio-mandibularis muscle, shown above to obtain in the Amphibia, but absent in all Teleostei which have hitherto been examined, is an illustrative example. The rela-

tions of these anterior cervical nerves are also more in accordance with their original distribution than are those of *Amia*, etc. Originally, one may suppose that the first and second spinal nerves were distributed to two distinct myotomes, the second also sending back a twig to unite with the third. On the modification and union of the ventral portions of these myotomes, concomitantly with the specialization of a pectoral arch, a greater portion of the nerve would pass back to the third, only a few twigs passing to the portion of the ventral musculature which represented the original myotome. This arrangement persists in the Urodelous Amphibia. In the Fish, however, the process went on still further, resulting in the disappearance of the twigs to the original myotome, the whole ramus ventralis passing backwards to unite with the third nerve.

On comparing what one finds in the Urodela with what obtains in the Anura, one finds that in the latter there is no nerve corresponding to the first vertebra; but the fact that even in the former the first nerve passes out, not in front of its vertebra, but through its arch, suggests a possibility of a still further retrogression having occurred in the Anura. This suggestion is, however, by no means sufficient to establish the fact. Let us examine the distribution of the nerves. The nerve which passes out in front of the second spinal vertebra in the Anura, after supplying the Mm. petro-vertebrales, passes downwards, and is eventually distributed to the maxillo-hyoideus, thoracico-hyoideus, and hyoglossus. It also sends a branch back to unite with the succeeding spinal nerve. It is very evident that the maxillo-hyoideus and thoracico-hyoideus are identical with the muscles of the same name in the Urodela, but apparently in the Anura the distribution of the nerve includes a muscle not mentioned hitherto. The hyoglossus is, however, absent, both in the Derotremata and Perennibranchiata, and in *Menopoma*, according to Fischer,[1] an unseparated part of the maxillo-hyoideus represents it. As to the petro-vertebrales, it is not easy to say what Urodelan muscles they represent, but perhaps they have been separated off from some of the shoulder muscles. Accordingly then, the distribution of this anterior nerve of the Anura corresponds exactly with that of the first and second nerves of the Urodela.

[1] As stated by Hoffman in *Bronn's Thierreich.*

Quite recently Ahlborn[1] has endeavored to show that the first three myomeres of *Petromyzon* are homologous with the three posterior cranial segments of the Anura. His mode of reasoning, however, is not conclusive, taking no account of the Urodelous forms which, as above stated, are less specialized than the Anura. The three anterior myomeres of *Petromyzon* are supplied by three dorsal branches from the so-called hypoglossal, *i. e.* one from each of its constituent nerves, so that these myomeres are the persistent portions of the original myomeres which have become modified and unrecognizable below, and form the "muscles of the tongue." Ahlborn bases his statement on the fact that the first spinal nerve in *Petromyzon* supplies two myomeres, the fourth and fifth, and since the true spinal nerve has disappeared in the Anura, therefore "können wir nur mit Sicherheit annehmen, dass der vorderste Spinalnerv der Anuren ursprünglich zwei Körpersegmente innervirt hat," therefore "der erste Halswirbel der Amphibien entspricht dem vierten Myocomma der Petromyzonten," and therefore "müssen die drei ersten Myomeren von Petromyzon, ... den drei hinteren Schadelsegmenten der Anuren homolog sein." It may be pointed out, that so far as Ahlborn's argument goes, the second spinal nerve of *Petromyzon* might with equal propriety be considered equivalent to the anterior spinal of the Anura, for it too supplies two myotomes, the fifth and sixth. From what has been already said, however, it seems evident that the so-called hypoglossus of *Petromyzon* is equivalent to the first spinal of the Teleostei, and this again to the first three spinals of the Urodela, which, in the Anura, are reduced to two, and therefore the first spinal nerve of *Petromyzon* must be equivalent to the fourth spinal of the Urodela and its representative in the Anura. In other words, the specialization of the first three spinal nerves has reached the same degree in *Petromyzon* and *Gadus*, but in the Amphibia the three original nerves remain distinct, or only the first and second unite.

When one turns one's attention to the Reptilia and the higher forms, one finds the arrangement somewhat different. Taking the Chelonia as an instance, one finds behind the foramen for the

[1] Ahlborn. *Ueber die Segmentation des Wirbelthierkörpers.* Zeit. f. Wissen. Zool. Bd. XL, Heft 2, 1884.

vagus, there is in the exoccipital another opening through which the hypoglossus passes. This nerve is distributed to the muscles of the tongue, speaking in a general way, and from analogy with the Amphibia there is little doubt but that these muscles are equivalent to those supplied by the first and second spinal nerves in the Urodela, and accordingly the hypoglossus of the Chelonia innervates muscles supplied by spinal nerves in lower forms. That the hypoglossus is here a true cranial nerve, and not the first spinal whose exit through the cranial walls is a secondary arrangement, is shown by the presence of a suboccipital nerve corresponding to the first vertebra. Other facts, as for instance the occurrence of a proatlantic ossification as described by Albrecht,[1] point to the same conclusion. One may decide then that the hypoglossus of the higher vertebrates corresponds to the anterior spinal nerves of lower forms, and is not a structure formed by a separation of the ventral roots of the vagus. As to the number of spinal nerves to which the hypoglossus of the Sauropsida and Mammalia corresponds it is difficult to say. The relation of the spinal nerves becomes changed to a very great degree as far as their distribution to the muscles of the fore-limb are concerned, so that in comparatively closely related forms the number and relative position of the nerves entering into the brachial plexus varies considerably, the variation being due, according to M. Fürbringer,[2] to the change of position backwards which the pectoral arch undergoes during fœtal life. The researches of Froriep,[3] however, on the developmental history of the hypoglossal in Sheep-embryos seem to indicate that the nerve is composed of as many as three primitive nerves, the author believing that he could distinguish, between the three bundles of fibres into which the nerve separated, traces of three distinct vertebrae. This point, however, I think cannot be considered perfectly decided, inasmuch as the nerve arises from the brain as a single root, and only divides into three later—an arrangement which is not usual in compound nerves, the peripheral portions

[1] Albrecht. *Ueber den Proatlas, etc.* Zool. Anz. Jahrg. III, 1880.

[2] M. Fürbringer. *Zur vergl. Anat. der Schultermuskeln.* III Theil. Morph. Jahrb. Bd. I, 1876.

[3] Froriep. *Ueber ein Ganglion des Hypoglossus und Wirbelanlagen in der Occipital-region.* Archiv. f. Anat. und Entwicklungsgesch. Jahrg. 1882.

of which unite first, the proximal parts later—and, further, only one ganglion was to be detected instead of three, as one would expect. Mayser's observations on *Cyprinus carpio*[1] appear to indicate that the hypoglossal of the higher forms is equivalent to only part of the first spinal nerve of the Teleosts. He says regarding the ventral root of that nerve, "Diese Wurzel ist mindestens in ihrem vordern Abschnitt ein Homologon des Nervus hypoglossus der Säuger; ihre Übereinstimmung mit dem entsprechenden Nerv der Reptilien und Vögel ist evident." From the distribution of the nerve it is impossible to say how many segments it represents, since the present state of our knowledge does not allow of a satisfactory relegation of the muscles of the higher vertebrates to their respective segments. It is probable that the hypoglossal represents two spinal nerves, but future researches will be required to settle this point.

In recapitulation one may imagine an ancestral form in which the vagus was the most posterior of the nerves issuing from the cranium, and behind it came a number of spinal nerves, each passing out in front of a corresponding vertebra. In the Ganoids the centra of certain of these anterior vertebrae ossified as one with the basioccipital, their arches and corresponding nerves then appearing to belong to the skull. In the Teleostei this commencing specialization was carried on in such a way that the vertebrae disappeared, and their nerves passed backwards to fuse with the next succeeding nerve, forming what is usually described as the first spinal nerve of these forms. In Petromyzon the specialization has reached a similar degree. In the Urodelous Amphibia the original relations are maintained, the vagus being the posterior cranial nerve, following which are a number of nerves each with its vertebra; in the Anura the first of these nerves passes back to unite with the second. In the Sauropsida and Mammalia, two, or perhaps three, of these anterior spinal nerves and their segments are incorporated with the cranium, the nerves forming the hypoglossus.

The cranium in the various orders of the Pisces and Amphibia is segmentally an homologous structure, but the cranium in the

[1] Mayser. *Vergl. anat. Studien über das Gehirn der Knochenfische mit besonderer Berücksichtigung der Cyprinoiden.* Zeit. f. Wiss. Zool. Bd. XXXVI, 1882.

Sauropsida and Mammalia is equivalent to an ichthyopsidan cranium *plus* two or three additional segments.

BALTIMORE, *Feb.* 7, 1885.

Since the above was sent to press, a second paper by Froriep[1] has come to hand, which, on account of its intimate bearing on some of the points referred to above, requires some notice.

The results I have obtained from the study of the arrangement and distribution of the anterior spinal nerves of various forms are confirmed in an interesting manner by Froriep's ontogenetic studies. He, from a further study of sections of sheep and cow embryos of various ages, has supplemented the facts announced in his earlier paper on the hypoglossal. He finds *two* ganglia on the embryonic hypoglossal; the nerve, however, apparently consisting of more than two portions, which eventually unite, the union occurring first at the origin of the nerve and proceeding centrifugally, and is quite convinced as to the homology of the hypoglossal of higher forms with the anterior spinal nerves of the ichthyopsida. He says: "Der Hypoglossus entsteht durch Vereinigung einer Anzahl segmentaler Spinalnerven, welche aus je zwei Wurzeln, einer ventralen und einer dorsalen, durchaus als Spinalnerven sich bilden."

As to the number of trunks united to form the hypoglossal, Froriep's researches indicate that there are three. He finds three muscle-segments in relation to the nerve, but has been able to discover only two ganglia, and the general arrangement of the nerve trunks is into two main portions, as a glance at his Fig. II will show. If there be really three muscle-segments supplied by the nerve, the presumption is strong in favor of the threefold nature of the hypoglossus; but until other structures which should be found in relation to the most anterior muscle-segment are discovered, such, for instance, as the ganglion, a definite conclusion must be withheld. It would seem clear that the hypoglossal in the Reptilia is composed of only two nerves, and I was inclined to believe the same with regard to the Mammalia. Our

[1] Froriep. *Ueber Anlagen von Sinnesorganen am Facialis, Glossopharyngeus und Vagus, über die genetische Stellung des Vagus zum Hypoglossus, und über die Herkunft der Zungenmuskulatur.* Arch. f. Anat. and Phys. Anat. Abth. Jahrg. 1885. Heft I and II.

knowledge of the homologies of the muscles of the anterior cervical and cranial regions of the Mammalia and Reptilia is too slight to allow any positive assertions to be made on the strength of apparent similarity of distribution of the hypoglossal in the two groups, and, until the homologies are made clear, embryological results must be provisionally accepted. If three nerves have really fused to form the hypoglossal of the Mammalia, it is interesting to notice that a similar fusion of the same number of anterior cervical nerves has taken place in three distinct groups, not at all directly connected by descent. In the Teleosts I have shown it occurs, and they must necessarily be considered as the climax of an unprecedented line of evolution; and in *Petromyzon* it also occurs, the group to which this form belongs being the culmination of degenerating offset from the main stem, bearing certain relationships to the Dipnoi. I believe the primitive Elasmobranchs, the Ganoids and the Teleostei to be connected together along one line, and the Cyclostoma, Dipnoi and Amphibia along another; the Dipnoi, however, having undergone a certain amount of specialization after the separation of the line to the Amphibia, as also the Elasmobranchs after the separation of the Ganoids.

One other result of Froriep's studies must be noticed. He finds the tongue musculature to arise from a ventral sheet of muscular tissue continuous behind with that from which the shoulder muscles are developed. This is exactly what was to be expected from what has been said above with regard to the origin of the tongue muscles in Amphibia. I showed that they were formed by a differentiation of a muscle corresponding to that known in lower forms as the Branchio-mandibularis, and this is on its part an anterior specialized portion of the hyopectoral. There has evidently been an extension forwards of the ventral muscular tissue belonging to one or more anterior post-cranial segments, a portion of which extension has developed into the tongue musculature, while the remaining posterior portion has formed the hyopectoralis and its derivatives in the higher forms.

BALTIMORE, *April* 28, 1885.

EXPLANATION OF PLATE X.

The bones have the following designations throughout:

Bbr_{1-5}	= Basibranchials.	Op	= Operculum.
Brst	= Branchiostegal rays.	Pa	= Parietal.
Fr	= Frontal.	Pal	= Palatine.
IOp	= Interoperculum.	Pfr	= Postfrontal.
Ju	= Jugal.	POp	= Preoperculum.
Men	= Submental plate.	Qu	= Quadrate.
Mn	= Mandible.	SO	= Supraoccipital.
Mx	= Maxilla.	SOp	= Suboperculum.
Na	= Nasal.	STp	= Supra-temporal

FIG. 1. Side view of skull of *Amia* after removal of integument, post-, sub- and preorbital bones and superficial portion of the Adductor mandibulæ. AM^1 = unremoved portion of superficial division of Add. mand. AM^2 = deeper division of Add. mand. LAP^{1-5} = various divisions of the Lev. arc. pal. GH = Geniohyoid. HH = Hyohyoid.

FIG. 2. Under surface of skull of *Amia* after removal of the integument and submental plate. IM = Intermandibularis. GH^1 = posterior portion of geniohyoid. GH^2 = Superficial portion of geniohyoid. HH = Hyohyoid. BrM = Branchio-mandibularis.

FIG. 3. Ventral branchial muscles of *Amia*. Iav_1^a = Interarcualis ventralis of first arch, anterior portion. Iav_1^b = Interarcualis ventralis of first arch, posterior portion. Iav_{2-5} = Interarcuales ventrales of second—fifth arches. BrM = Branchio-mandibularis. PhC_1 = Pharyngo-clavicularis Ext. PhC_2 = Pharyngo-clav. int. Tva = Trans. vent. ant. Tvp = Trans. vent. posterior.

FIG. 4. Diagram of the branches of the Trigeminus *a* and *b* = branches to first division of Lev. arc. pal. Bu = Buccal branch. RMS = Ramus max. super. RMI = Ram. max. infer. *c* = branch to fourth and fifth portions of Lev. arc. pal. *d* = branch to Add. mand. *e* = cutaneous branch. *f* = branch to geniohyoid. Fac = Ram. mand. ext. facialis.

FIG. 5. Diagram of the branches of the facial. Rop = Ramus opercularis. RM = Ramus mandib. RHy = Ram. hyoideus. RMI = Ram. mand. intern. RME = Ram. mand. ext.

ON THE ENDINGS OF THE MOTOR NERVES IN THE VOLUNTARY MUSCLES OF THE FROG.

By CHR. SIHLER, M. D., Ph. D., formerly Fellow of the Johns Hopkins University. With Plate XI.

In the following communication I wish (1) to describe a new method of demonstrating the nerve-endings in the muscle of the frog, and (2) to bring forth evidence supporting the view that the terminal nerve-fibres are situated on the outside of the sarcolemma, and do not, as is taught by most authorities, penetrate this envelope.

Even if the method here employed and described should not bring out any further points than have been already demonstrated, in other ways it would seem worthy of being brought to the notice of those interested, because it is not a difficult one, and because it furnishes specimens which can be mounted and preserved indefinitely.

That both of these points are desiderata we can see from Kühne's article in Stricker's Handbook. He says, in speaking of the methods employed in investigating the nerve-endings, that this is one of the most difficult departments of microscopical technique, and therefore one in which microscopists have not yet reached uniform conclusions; that the muscle-fibre used for examination must be taken from irritable muscle, and care must also be taken that the isolated fibres, while under the cover-glass—which, moreover, must not be allowed to press on the fibres—have retained enough vitality to be still able to contract.

The gold method is very complicated, and those who have used it speak of unaccountable failures, and, moreover, the specimens soon change. The same may be said of the silver method, which Kühne commends because it yields specimens which can be preserved for at least a few months.

I have called the method here presented a new one, but must qualify this statement, inasmuch as it is essentially the method of Beale, although, as will be seen, the results reached by the writer differ entirely from those of Beale, who, as is well known,

claims that the motor-nerves run into a fine network which envelopes the muscle-fibres. The method of Beale has this great advantage, that it preserves the natural condition of the tissues without making them brittle and hard, and thus allows them to be handled very roughly, and further, that it yields very clear specimens.

After trying in vain for some time by Beale's method to find the nerve-endings described by authors, I resorted to the modification hereafter described, and obtained a most satisfactory result. In working with Beale's fluid I found that, as a rule, such substances as the axis-cylinder and striped and unstriped muscle would not stain at all, or only slightly, but that occasionally some of the nerve-fibres would be found which had taken up the coloring matter, and that thus specimens would be obtained which were very satisfactory as showing much and showing that plainly, and in not cutting off as much light as haematoxyline specimens are apt to do. My aim, therefore, was to procure a red staining-fluid which would be a little more active than carmine dissolved in aqua ammonia and glycerine, and which might thus pick out the fine nerve-fibres. I therefore ground up one-half ounce of cochineal, added one fluid ounce of aqua ammonia, three fluid ounces of water and four fluid ounces of glycerine, set aside this mixture for some weeks, shaking it occasionally, then filtered. To the filtrate I added twenty grains of carmine dissolved in aqua ammonia and water, and then boiled until the smell of ammonia was no longer perceptible. Finally, dilute alcohol was added to make up eight fluid ounces.

I then injected a frog with Turnbull's blue suspended in glycerine and water as recommended by Beale, because, even if the injection is imperfect, it helps to analyse the specimens; removed the skin from the extremities; placed these in the staining-fluid in a dish with a lid which did not close very perfectly; and set them aside for some time—I cannot state how long, certainly several weeks. It was in the summer. Of course, no definite time can be given; temperature, and I do not know what other conditions, influencing the rapidity with which tissues become stained. It is well to examine some of the material every two or three days, to find out when the process has continued long enough: it ought to be continued for a rather long

period of time. After the material had become thoroughly and deeply stained, I placed it in a fluid consisting of about fifty parts (by measure) of glycerine, twenty-five of water, twenty-five of alcohol, and one-half to one part of acetic acid. The tissue was allowed to remain in this fluid, which was renewed every other day for about a week, and finally was preserved in a similar fluid, which contained, however, a still smaller amount of acetic acid. The acetic acid clears up the tissue, and by softening and partly dissolving the connective tissue which holds the muscle-fibres together, aids in separating and isolating them. The alcohol when thus diluted does not make the muscle-fibres too brittle, and yet hardens them enough, so that they can be handled and teased without difficulty.

Some of the material thus treated will be found to be stained too deeply, and parts not stained enough, but other portions will be just right. It happens that in some parts the muscle-fibre with its nuclei will be stained comparatively slightly, while the nerve-fibres and nuclei have been colored very deeply. These latter yield the most beautiful and perfect specimens, and after one has become familiar, in these more perfect specimens, with the appearance of the structures concerned, he can then discriminate them in specimens which are less perfect.

Overstained fibres may be cleared up by placing them, for a longer or shorter time, in mixtures of acetic acid and glycerine of varying strength. Hydrochloric acid is still more active; very weak solutions, only one-tenth to five-tenths of one per cent., are to be used. By such treatment specimens will become clear and show beautiful nerve-endings, where before the general redness allowed no discrimination of the elements. It must, however, be remembered that specimens thus treated are apt in time to bleach still more, and it is doubtful whether the most careful removal of the acid will insure their permanency.

The muscle-fibres are to be teased in glycerine. A small bundle of untorn fibres is extended at full length between two slides and subjected to slight pressure with the fingers. The fibres will thus be more easily separated without breaking. Low powers—about 200 diameters—will give very satisfactory views, and allow the necessary manipulation for the separation of the nerve-ending from the muscle-fibre. Placing the muscle-fibre in

alcohol before teasing has this advantage, viz. when thus treated the fibres are more readily separated, but has the disadvantage of hardening the fine nerve-fibrils and thus shrinking them; it also seems to have the effect of contracting and shrivelling the connective tissue, thereby pulling the nerve-fibres which are enveloped therein, off from the muscle. This is a disadvantage, if one is looking for the nerve-endings *in situ* upon the fibre, but is of advantage if one wants to show that the terminal filaments of the nerve can be stripped off from the muscle-fibre.

My observations have not been extensive enough to say what muscles will give the best results. I began with examining the very short ones, but found that longer fibres, *e. g.* those from the fore-arm, yielded more satisfactory specimens. The size and development of the nerve-endings seem certainly to correspond with the size of the muscle-fibre. Thus the typical nerve-ending figured in the books was not to be found on the short interdigital muscles of the extremities.

The plate accompanying this paper presents drawings of four nerve-endings *in situ*. Figures 1, 2, 3, 4 show that I have really found the nerve-ending, and I think that the method employed brings out all the points shown by the figures of Kühne, Kölliker, Klein, Arndt, and more than that of Ranvier, from specimens prepared by the gold method. By comparison of their plates I find that fibres figured by Kühne are broad in comparison with the nuclei, at times exceeding them in width, while Kölliker's fibres are narrower, as compared with the nuclei. The figures on the plate most resemble those of Arndt in Kirke's Physiology; the fibres are narrower and the nuclei are larger (as compared with each other) than as represented by Kühne or Kölliker. The four figures present somewhat of a variety; while figures 1 and 2 may be looked upon as rather typical, figure 4 is somewhat of an exception. By a little change in the arrangement of the nuclei, *i. e.* if they were arranged in a circle instead of in a row, something like the end-plate of the mammalian muscle would be formed. Fig. 3 shows that the fine fibrils may run at right angles to the muscle-fibre, although the parallel course is the more common.

As regards the description of the nerve-ending nothing new has been brought to light. The medullated nerve-fibre generally

has but little of the medullary sheath about the part near its attachment to the muscle-fibre. It is, however, often richly supplied with nuclei, which are frequently of large size. Sometimes its course runs for some distance along the muscle-fibre, but in other instances it breaks up into its terminal fibrils as soon as it touches the muscle-fibre. Generally the change is a sudden one, the main fibre retaining its thickness and sending out a number of fine fibres from its end, while more rarely there is a gradual change, the medullated fibre growing thinner and thinner till the diameter of a terminal filament is reached. The form of the nuclei belonging to the fine end-fibrils is somewhat characteristic; they have a tendency to roundness, being pointed a little where the fibril enters and leaves them, although oblong nuclei are quite frequent; rarely are they as pointed or spindle-shaped as the nuclei of the nerves supplying the capillaries. The peculiar form of the nuclei and the depth to which they often are stained aid in finding the fine fibrils running between them and from them. The fine end-fibrils, especially their terminal portion, often appear as a double red line. Does the fibril consist of a delicate envelope enclosing a rather soft protoplasm, which by coagulating separates into an outer denser part, which absorbs the coloring matter and an inner fluid part? In that case the end fibre would not be a naked axis-cylinder. The fibrils generally end in a point, but are at times a little rounded. Certain it is that this is a real ending and not an appearance artificially produced, so that Beale's view is not correct. Beale certainly did not, as Kölliker suggests, take connective-tissue fibres for nerve-fibres. I should rather think that the nerves he shows are the sensory nerves of the muscles.

The peculiar form of the nuclei, their large size compared with the fibre, and the double red line of the fibrils, are very characteristic; and are important in settling the question whether the nerve-ending is beneath or on the outside of the sarcolemma.

Now the question where the endings are situated seems to be of some physiological importance, because there will probably be a difference in the way in which the nerve-current calls forth the contraction of the muscle, if the nerve and muscle substances are placed in immediate contact on the one hand, or if on the other the nerve has to influence the contractile substance through the

intervening sarcolemma, and possibly through a sheath surrounding the fine nerve-fibril also.

This question seems to be also of importance for another reason. Striped muscle certainly exhibits centrifugal nerve-endings on the largest scale, and we naturally and with propriety make inferences from the arrangement we there find to structures more minute, which are difficult if not impossible to analyse, *e. g.* the nerve-endings in or upon the unstriped muscle-fibres.

On this question Kölliker says: "Krause, Rouget and myself place the end-fibre altogether on the outside of the sarcolemma; Kühne, Engelmann and Waldeyer [he might have added several other names, *e. g.* Klein, Gerlach, Frey] however, on the inside, and there is no doubt that this point is a very difficult one to decide . . . . . . According to Kühne the *whole length* of the pale nerve-ending is situated within the sarcolemma; but I have demonstrated, and Krause supports this view, that many pale fibres are situated on the outside of the sarcolemma. Regarding the *ultimate ends* of the pale fibres, however, it seems almost impossible to give a definite answer, whether they are situated within or without the sarcolemma, because the delicacy of the tissues in consideration makes accurate observations almost impossible."

The following observations, made on material prepared by the process described above, indicate, or rather demonstrate, if accurate, that the whole of the end filaments of the motor nerves are situated on the outside of the sarcolemma.

1. Frequently when an end-fibril is seen to run along the side or edge of a muscle-fibre, *i. e.* is viewed, so to speak, in profile, the nerve-nuclei will be seen standing out in marked relief from the muscle-fibre.

2. It sometimes happens that the contents of the sarcolemma are displaced by pressure of the needle, or some other accident in the manipulation, while the nerve-ending is scarcely disturbed. Figure 5 shows this; all the muscle-substance proper had disappeared from beneath, and yet all the details of the end fibrils can be clearly seen; there is no disarrangement or breaking of the filaments, except that they are dragged a little out of their straight direction. Now it would seem rather curious that such perfect preservation of the naked protoplasm of the fine fibrils

could be obtained while the muscle substance in immediate contact was thus pushed aside. Of course, these observations go to support Kölliker's view.

3. I have prepared quite a number of specimens, where several muscle-fibrés were lying side by side, and one of the ultimate nucleated fibrils passed from one muscle-fibre over upon the other. Figure 6 shows one of these. Here we see a fine nerve-fibril passing from one muscle-fibre across a second and over to a third one.

To this observation the objection may be made that this arrangement is rather exceptional; yet it is of some value, and in very short and thin muscle-fibres, such as we find in the hand and foot, this passing of the terminal fibre from one muscle-fibre to the other is probably not so rare, although it is difficult to get fully satisfactory specimens.

4. Not very unfrequently, specimens are obtained in which the ending is seen detached to a varying extent. Figs. 7, 8, 9, 10, 11 show this, and others might have been added. In all these cases the separation was an accidental one, brought about during the separation of the muscle-fibres. The number of times this condition has been found, and the variety in the appearance as shown by the drawings, prove that no accidental deception has come into play. The partial attachment of the nerve-fibres also shows that it is the nerve-ending belonging to that particular muscle-fibre. The figures show various degrees of separation; *e. g.*, in Fig. 8, the whole ending is displaced, and it is not easy to tell what the arrangement before displacement may have been. In Fig. 11 the muscle was supplied by two main fibres, the ultimate ending of one fibre is detached, as is also a portion of the other fibril, where the muscle shows a depression. In all these cases the nerve and muscle, to which the nerve-ending belonged, were together. Repeated instances were found, in examining teased muscle, of cases where the nerve-endings were wholly separated from the muscle-fibre. I regard the double red line and the large size, together with the form of the nucleus, as so characteristic of the end-fibrils that such structures can be confidently called detached nerve-endings.

5. With some patience and proper specimens I have succeeded a number of times in isolating or separating the nerve-ending

from the muscle-fibre. The specimens which yield such results are those in which the nerve-fibre approaches the muscle from the side, where the former is not too short, and where just the right amount of connective tissue envelopes the nerve. The separation was produced by pressing, intermittently of course, the cover-glass with the needle, and by pushing it from side to side in the direction of the muscle-fibre, as well as at right angles to it and obliquely. I have done this while the specimen was under actual observation through the glass. Sometimes we thus manage to grasp the medullated fibre, which of course must project a little from the muscle-fibre to which it is attached, and to pull or peel off, if not the whole nerve-ending, often half of it, the main fibre first becoming detached and then the fibrils; all, of course, protected and surrounded by the gelatinous connective tissue which holds the muscle-fibres together. Nothing can have such a strong influence in forming one's opinion as to the situation of the nerve-ending as having performed this operation. As a matter of course the whole of the ending cannot always be detached; the fine fibrils generally pass off in opposite directions, and while the main or medullated fibre is dragged in one direction to peel off one set of filaments, those filaments running in the opposite direction will be apt to be torn from the main fibre and remain attached to the muscle-fibre. But this point is certain, viz., that a terminal fibril can, with careful manipulation, be detached throughout its whole length, even down to its very end.

This manipulation can be more readily performed if the muscle-fibre be first treated for a short time with acetic acid or with weak muriatic acid. These reagents seem to swell up and soften the substance surrounding the muscle and nerve-fibres, and thus aid in separating them.

I must not forget to mention a little manœuvre which I have found very useful. It is to bend the muscle-fibre, to which a nerve-ending is attached, in such a way that the nerve-ending comes to be placed in the concavity. Then pressure upon the cover-glass will pull the nerve one way and the muscle the other; and thus I have made a number of successful separations.

From the foregoing observations we are, I think, justified in the conclusion that *the whole nerve-ending is situated on the outside*

of the sarcolemma, and, like the capillaries, imbedded in the gelatinous connective tissue.

Figs. 12, 13, 14, 15, 16 show nerve-endings which were purposely detached.

In Fig. 15 the whole nerve-ending is detached; as this belonged to a small muscle-fibre, and hence was not very elaborate, it could be wholly detached.

I wish to call special attention to Figs. 13 and 14, because the specimens gave evidence showing the detachment of the very extremities of the fine end-fibrils, by their forking, which is a peculiarity not mentioned above, but very characteristic. Fig. 14 was drawn while the process of separation was in progress. Here the extremity of one fine fibril at (*a*) was separated, while the main mass at (*b*) was still adherent. Fig. 13 also shows the same forking.

I dwell on the point that *the fine fibrils can be separated in their whole length, their very tip included*, because Kölliker, who also asserts that the beginning at least of the fine fibrils is on the outside of the sarcolemma, admits, in the quotation given above, that he has no testimony to offer as to the remaining parts of the fibrils.

In Fig. 12 the broad fibres are wholly detached, and the fine fibrils partly; and a little more manipulation would no doubt have made the separation complete. One dislikes to continue the process too far if a good specimen for a drawing is desired, because one will be apt to destroy what has been gained, by crushing or tearing the parts.

The preparations which show the separation cannot, I am sorry to say, always be preserved. The cover-glass is at times after the manipulation in such a position on the slide that the specimen cannot be mounted, as any change either in position or pressure will disarrange the parts. Again, the elastic connective tissue which envelops the nerve-ending, and which still may hold muscle and nerve together, tends after their separation to draw the latter back to its old position, so that on re-examining your detached nerve-ending after some days you may find it returned to its old position, in contact with the muscle-fibre. This happened in the specimen from which Fig. 12 was drawn.

This method of staining also brings out beautifully the fine nerves (probably sensory) in the frog's tongue, and likewise the nerve-supply of the vessels, especially of the capillaries; and it is a fact which seems not to enter sufficiently into our physiological conceptions, that the nerve-supply of the capillaries of such tissue as the striped muscles is simply enormous as compared with that of the muscle-fibres themselves.

I repeat also that all the manipulations must be carried on in glycerine.

In conclusion, I would say that the separation of the nerve-endings takes a good deal of patience and some time; there will be many failures; certain muscle-fibres only are suitable, and some of these will not allow the detachment of the nerve-ending; but, on the other hand, the rough handling which tissues prepared as above described will stand is also remarkable and encouraging.

Kühne's categorical statement that Kölliker's view is all but abandoned (allgemein verlassen) gives me little hope of being listened to, but on account of the physiological importance of this question I have taken the liberty of bringing my work to the notice of investigators in this branch, for the purpose of calling attention to the method here described, and inviting those who have the time and patience to take up the subject again.

CLEVELAND, O., *March*, 1885.

DESCRIPTION OF PLATE XI.

Figs. 1, 2, 3, 4. Nerve-endings on muscle-fibre in situ.

Fig. 5. Nerve-ending on empty sarcolemma.

Fig. 6. Fine nerve-fibre passing over several muscle-fibres.

Figs. 7, 8, 9, 10, 11. Nerve-endings found more or less detached during process of teasing.

Figs. 12, 13, 14, 15, 16, 17. Nerve-endings separated by manipulation of cover-glass.

Material used, frog's muscle.

Power used, Gundlach's objective III. Eye-piece III, about 175 diameters. Size of drawings reduced.

Muscle nuclei: connective tissue and its corpuscles omitted.

MARINE LARVÆ AND THEIR RELATION TO ADULTS.

By H. W. CONN, Ph. D., Instructor in Biology at Wesleyan University. With Plates VIII and IX.

Some time ago I had the opportunity of making a study of the early history of the Thalassema larva, and the results obtained were so suggestive as to lead me to an extensive study of larval forms in general, to see if the indications here hinted at were of any general importance. As I have already indicated[1] I have been led to conclusions partly in accordance with Balfour's theory of larvæ, which is the most obvious conclusion resulting from the study of Thalassema larva.[2] The publication of Sedgwick's paper,[3] and that of Wilson,[4] have led me to put my views into shape, in order to show what are the teachings of larvæ upon some of the general questions of these two papers. The following discussion, therefore, will in part be a testimony to Balfour's views, in part will differ from them; while I have endeavored to carry the views farther, and indicate what are the teachings of larvæ as to the relations of adults.

I have first endeavored to hunt for the simplest type of larva known, which is at the same time most universally included in the development of animals. And having found such a larva, I have endeavored to trace its relation to the various known existing types of larvæ, and hence to the adults, and this plan I shall follow in the present paper.

Of all the larvæ with which we are acquainted, the form found among certain Nemertians, and known as the pilidium, is undoubtedly the simplest which can really be called a larva (Fig. 1); and not only is it the simplest, but, as we shall have reason to conclude, it is probably the most primitive. Since the formulation of Haeckel's gastrula theory, evidence has been accumulating on every

[1] J. H. U. Circular No. 22.

[2] See a paper on Thalassema in these Studies, Vol. III.

[3] A. Sedgwick: Origin of Metameric Segmentation, Quar. J. Mic. Soc. 1884.

[4] E. B. Wilson, Mesentereal Filaments of Alcyonaria. Naples Mittheil. 1884.

hand to show that this theory expresses in some sense a truth; for the more we know of the history and structure of animals, the more certain is it that the gastrula stage is included in the ontogeny of all animals. We may therefore justly assume it as a starting point. Now the Nemertian pilidium is little more than a gastrula, and in this there is reason for thinking it a primitive form. But the pilidium, however, is not a simple gastrula, for it has two features which the gastrula is not supposed to possess. First, there is usually present at the extremity opposite the mouth, or, as it will be preferable to call it, the blastopore, a certain tuft of cilia, and an accompanying ectodermal thickening. This tuft of cilia may consist of a single long cilium, it may consist of several, or it may consist of a large number; but in all cases they differ from the remaining cilia of the body, being of different length and thickness, and never truly vibratory, and therefore not locomotor organs. Usually we find them carried quite stiffly, protruded in such a manner as to indicate that they are sensory organs. At all events they do indicate that this region of the body has become differentiated from the rest.

Secondly, there is found around the border of the bell of the pilidium a special band of cilia. The whole larva may be covered with cilia, but around the border of the bell they are much longer than elsewhere, forming a band which serves as a locomotor organ, enabling the animal to swim about with a much more regular motion than would be possible with a uniform covering of cilia. Here, too, is usually seen an ectodermal thickening, which is made of cells of different character from the rest of the ectoderm, and may occasionally, indeed, contain sense organs.[1] Evidently we may be sure that here is a second differentiated region of the ectoderm where the cells have acquired special function. In our pilidium larva, therefore (Fig. 1), we have a gastrula with an aboral tuft of sensory cilia, and a circumblastoporal ring of long locomotor cilia, both tracts accompanied by an ectodermal thickening.

And now the question arises as to the meaning of the ciliated bands,—whether we can draw any conclusions from them It is difficult to say just how much confidence can be placed upon the presence of cilia as an evidence of relationship. Cilia seem to

[1] Wilson. Pilidium larva. In these Studies, Vol. II.

be about the simplest organs with which animals can be endowed, and are, moreover, the most widely distributed. In studying larva, we almost universally find cilia developed in abundance; but this fact is not the slightest evidence for believing in any relationship among the larvæ in question, but seems rather to be reason for thinking that cilia can be of no value as indicating relationship. Indeed it seems to be a general property of protoplasm to develop cilia whenever and wherever they are needed, and we consequently find them almost everywhere. We may find a larva completely covered with them, and another with a number of definite bands, but always cilia in some form; they are found in Infusoria, they are found in Vertebrates. It is evident, therefore, that great caution must be used in basing any theoretical conclusions as to relationship upon the presence or absence of cilia. A uniform covering of cilia has probably no phylogenetic meaning, and the fact that various larvæ arise from a uniformly ciliated condition has not the slightest significance.

But, on the other hand, it is impossible for one who has made any extended study of a large number of larval forms to avoid the conclusion that certain definite conclusions may with surety be based upon some of the *tracts* of cilia. Take for instance the tuft of sense cilia above described. If we find such a tuft of cilia almost universally present among free swimming larvæ, and always with the same relation to the rest of the body, we can but draw the conclusion that this tuft has more than accidental significance. Now this ciliated tuft is of very wide distribution. It is found in the free swimming larvæ of Coelenterata, it is almost universally present in Annelids and Nemertians; it is found in Planarian larvæ; is present in Mollusks and among Polyzoa and Brachiopods (?); and is also, as is shown by some careful observations of Mr. Nachtrieb, in a paper to be published soon, found in Echinodermata; and in all these forms it has a similar relation to the rest of the body, though not always with similar appearance. A structure of such wide distribution must certainly have considerable meaning in the phylogeny of larvæ, if any conclusions in this regard can be based on ontogeny. The same may be said in regard to the ring of cilia around the blastopore which is almost as widely distributed (the Echinoderms are perhaps an exception), and, as I shall attempt to show, with similar relations

in all forms. From the mere presence of cilia, locomotor or sensory, no matter how widely distributed, no conclusions as to relationship can be drawn; but when we find almost universally among larvæ which show other relationships to each other, a certain very definite band of cilia accompanied by an ectodermal thickening, and always bearing similar relation to the body, we are certainly justified in considering that we are dealing with something of more meaning than that of a general development of cilia.

Finding them so widely distributed, therefore, we are justified in believing that this sensory tuft and this locomotor band in our pilidium have some distinct meaning. But what is this meaning? Are we to assume that these two regions of the larval body are set apart, the one to bear sensory cilia and the other to bear locomotor cilia? I think we cannot rightly draw such conclusions, nor even conclude that these two regions originally and necessarily bore cilia. In the first place, we get certain suggestions from the history of the sensory ciliated tuft in Annelids. They always arise in connection with an ectodermal thickening, a thickening which, in Annelids, becomes the supra-oesophageal ganglion. From the cells beneath the cilia, indeed from the very cells which give rise to them, is developed that part of Annelid's nervous system which is the seat of the sensory functions, and we have, therefore indications that the cilia in question mark the position of an already partially differentiated nervous system. The same conclusion is indicated by the already mentioned fact of the great variation of the character of this sensory tuft, composed now of a tuft of long motionless cilia, now of a few short ones, or again of one very long hair. This fact alone would convince us that it is not the cilia themselves which are important, but the cells from which they arise. It teaches us that we are dealing, not with cilia alone, but with an already partially differentiated nervous system. The cerebral ganglion has in fact even now made its appearance, just at the close of the gastrula period.[1]

[1] This fact is certainly a strong argument against all views which make the cerebral ganglion of Annelids a part of the same nervous system from which the ventral chain arises. Sedgwick, in making the cerebral ganglion the anterior extremity of a single nervous ring, seems to have overlooked, or at least not

With regard to the circumoral band of cilia, there are many facts which point to a similar conclusion. Wilson has shown that in one species of pilidium, sensory organs may appear around the bell margin.

Here we usually find pigment developed. The cells of this band are usually of a different character from those of the remaining ectoderm. In Thalassema and Serpula, which I have most carefully studied, this band goes through two markedly distinct stages. During the first two days of larval life the ring consists of a broad band of cilia which are quite powerful. Such it remains until about the third day, when it is replaced by something entirely different. The broad band of cilia disappears, and there comes in its place a single row of very long, powerful cilia. This row occupies the same position as did the original band, and has an entirely similar function; but it is evidently not the same, and cannot be considered as exactly homologous with it. What we have in Thalassema and Serpula therefore is a special band of ectodermal tissue which has become differentiated from the rest and gives rise to two entirely independent bands of cilia. All of these facts are enough to show that it is not the band of cilia which is of importance, but the band of ectodermal cells which gives rise to them.

These few facts give us some hints as to what meaning we are to put on the various ciliated tracts. They do not indicate that because various larvæ possess a preoral ciliated band, these bands are necessarily homologous with each other. They may be, and probably are, independently developed in many cases. But they do indicate that even in our early pilidium larva as well as in all other larvæ where this tract is represented, there is present a certain tract of ectodermal tissue which has acquired a function different from that of the rest of the ectoderm, a tract which may give rise to cilia, or sensory organs, or tentacles. The sensory tuft of cilia shows us that thus early in the larval

mentioned, the fact that it always arises as a separate portion in all Annelids and Arthropods, and is only at later periods united with the ventral chord. The same difficulty is seen in accepting Dohrn's view, which makes the cerebral ganglion homologous with part of the vertebrate brain, for the vertebrate brain always arises as the anterior part of the spinal cord, and never as a separate organ. This difficulty has never been sufficiently answered.

development a certain region, a nerve plate (Scheitelplatte), has become differentiated as a region of special activity; a region which we know to be nervous, since the cerebral ganglion of the adult is developed from it.

Now as we look again at our pilidium larva we get further insight into its structure. It is not an undifferentiated body enclosing a digestive sac, *i. e.* a simple gastrula. It consists rather of an animal which possesses mouth and digestive tract, and has already definitely indicated, though of course as yet very slightly differentiated, two ectodermal tracts which we may, provisionally at least, call nervous. One which bears a tuft of sensory cilia is undoubtedly nervous, and already indicates that part of the body which is to become anterior end; while the other, which is probably also nervous, forms a ring dividing the body into two unequal parts, a larger one in front and a smaller one behind. These two regions I will call for convenience the preoral and oral lobes; the former including all in front of the ciliated band, the latter including all upon the other side of the band and enclosing the blastopore.

A word is also necessary upon the value of larvæ as indicating relationships as compared with egg embryology. Valid reasons present themselves against accepting evidence from either source. It is urged that larvæ are free swimming independent animals, and that they have undoubtedly been modified by their conditions. If this is the case we cannot assume that they will preserve with any degree of correctness the primitive history of the groups of animals to which they belong. The necessity for locomotion and capture of food will introduce secondary changes and greatly modify the history. On the other hand, it is just as legitimately urged that the egg embryos are also greatly modified by their change of condition. They have lost the locomotion which the early forms must have possessed, and are, moreover, supplied with a store of food; which two facts induce at least as great changes as have taken place in free forms. All locomotor organs which the ancestral forms possessed will be lost, the digestive system will be modified, and there will be no longer a need for a mouth, etc.

Undoubtedly both of these positions are well taken, and neither egg embryology nor larval development can give us an

unmodified history. Each will, however, to a certain extent, offset the other. That development will teach us something, no one will be inclined to deny; but the true teaching of development will come from the union of larval history and egg embryology. No rule can be given; and if egg embryology and larval history come in direct conflict, we cannot say beforehand which, if either, must be accepted as the least modified. Each case must be examined by itself, and conclusions be drawn as to which is the more trustworthy in special cases.

In the cases which we are to consider I think there can be no doubt as to the fact that the free larvæ are more primitive than the egg embryos, as far, at least, as concerns the early stages. Larvæ are unquestionably in circumstances which are more like those of the early ancestors than are those of the egg embryos, for they are free and independent, must procure and digest their own food, all of which characteristics they must share with the ancestral types. Other things being equal, therefore, they will retain in their general features something of the ancestral form. Can there be any doubt, for instance, that the history of Serpula is more primitive than that of Arinicola? Compare the free larva of the former (Fig. 13) with the egg embryo of the latter (Fig. 15). In the former we have an independent form resulting from a regular segmentation and invaginate gastrula; in the latter, an embryo with no digestive system, which has resulted from an epibolic gastrula, which no one claims as primitive. Compare the development of Polygordius with that of Nereis, and there can be no doubt as to which is the more primitive.

Moreover, if larvæ do not retain ancestral features there is no reason why they should show any great resemblance to each other. In some few general features we should expect that they would be alike, owing to their like conditions. All being swimming animals, we should expect the presence of cilia; all being independent, we should expect a functional digestive system; all being marine forms with many enemies, we can explain their almost universal transparency. But when we come to deal with more specific features, any such explanation fails. That all larvæ should possess cilia is easily enough understood, even if we do not believe in any phylogenetic rela-

tion between them; but that all larva, or a large majority of them, should possess a definite band, quite variable in appearance, but always preserving its fundamental relation, is not to be explained in any other way than by phylogenetic inheritance; for otherwise this band would be independently acquired in a large number of cases, which is as difficult to understand in larvæ as in adults.

There is no reason then why larval characteristics, provided that we can select definite ones of wide occurrence, are not as valuable to indicate relations as are those of the adult, and perhaps more so than those of the egg embryo. I have therefore endeavored to select such as are almost universal, and shall take into consideration chiefly the two ectodermal tracts above mentioned, which I think can be shown to be present in all larvæ. Various other features could be selected here and there, but none of such wide significance as these two, and I have therefore thought it well to leave them out of the question.

Starting now with our pilidium form, we first ask how widely distributed is this stage? Do all larvæ pass through anything equivalent to the pilidium? This question cannot be strictly answered in the affirmative, but it is much nearer true than I was at first inclined to believe. The fact that the pilidium is hardly more than a gastrula will show that all larvæ, and indeed all animals, include in their development a stage which is very nearly a pilidium; but the fact that it *is* more than a simple gastrula shows that it is probable that some forms do not, and perhaps have never possessed all its characteristics. But we do find it, however, very widely distributed. The Coelenterata in many cases possess a very typical pilidium form. Fig. 2 is an unknown Actinian found at Hampton, which, while not having a circumblastoporal ciliated ring, does have the ectoderm thickened in this region. It is therefore a true pilidium. The Annelids, as is shown in Fig. 10, possess a typical pilidium stage, and the same is true of most of the group of worms which possess free larvæ. The Molluska usually have their early history much modified by the presence of a large amount of food yolk, but Blütchli's figures of Paludina, a form with little food yolk, will show that a true pilidium stage is also found in this group. Polyzoa pass through the stage, as an examination of

Hatscheck's figures[1] will abundantly show. Echinoderms have never as yet been considered to possess any similar stage, nothing like a preoral sense organ having been until recently described. But I have been pleased to learn this is due to want of more careful observation. Mr. Nachtrieb[2] has found that a careful study of the ciliation of the young Echinoderm reveals, in many cases at least, a well developed anterior tuft of cilia, and an ectodermal thickening identical in appearance with that of Serpula, Fig. 10. The circumoral ciliated band is, however, not evident. This interesting discovery of Mr. Nachtrieb has placed the Echinoderms in the same category as that of other larvæ, at least as far as the preoral sense region is concerned.

On the other hand, of course, Sponges and Arthropods show no approach to anything like our pilidium larva; but these animals possess a larva so different from the ordinary forms we are studying that they have no significance for us here. Rotifera, Brachiopoda and Enteropneusta are not as yet sufficiently known in their development to make it certain whether a true pilidium stage is included or not. All of the larvæ, therefore, which are open for our discussion except the Echinoderms, pass through a true pilidium stage, and the Echinoderms have a stage very much the same.

The next question arising is as follows: Do we find anywhere in the animal kingdom this type preserved as an adult form? The answer is, that while we do not find it in its typical form, some Coelenterata do present for us a form which with little modification is a pilidium. This can be best seen by studying the history of some form with a free larva. Actinia being well known from the work of Lacaze Duthiers,[3] is best adapted to our purpose. The Actinia larva, comp. Fig. 2, corresponds well with the pilidium form. It is a gastrula with mouth at one extremity and with a sensory tuft at the other, having around the blastopore an ectodermal thickening, though not a particular row of cilia. This difference is of no importance, for we have seen reason for

[1] Hatscheck. Embryonal ent. a Knosp. d. Pedicellina echinata. Zeit. f. Wis. Zool. xxix.

[2] J. H. U. Circular, 1885, No. 38.

[3] Arch. f. Zool. Exp. et Gen., vol. .

believing that the circumblastoporal band simply indicates ectodermal differentiation, and this does undoubtedly exist in Actinia, as is shown first by the thickened ectoderm, and secondly, by the fact that this region soon buds out tentacles. The conversion of this larva into the adult is an exceedingly simple matter. It attaches itself by the aboral extremity, *i. e.* by the extremity which carries the tuft of sensory cilia. Once attached, it is nothing more than a stationary pilidium, with the exception that the circumblastoporal ring develops tentacles instead of cilia. And this fact need not surprise us, for if, as we have seen reasons for believing, the circumblastoporal ring is simply a ring of differentiated ectodermal tissue, we may as well expect tentacles as cilia, or very likely both, as may be found in certain Actinula. In Actinia, this simple attached pilidium undergoes quite a number of later changes, such as the formation of mesenteries and stomadaeum, so that the adult is quite highly modified, and can hardly be compared with the pilidium. But having now found out the relation of the adult to the larva, it becomes immediately evident that in Hydra we have simply an attached and stationary pilidium. It is true that the sensory ciliated tuft has disappeared, but this is, of course, necessary with the attachment by its aboral extremity. The circumblastoporal nervous tract no longer having use for locomotor cilia has replaced them by sensory and prehensile tentacles. But these changes do not materially change its identity with the pilidium form.

It may be objected that this view of the Hydra and Coelenterata makes cilia homologous with tentacles. But this is an erroneous understanding of the subject. To my mind, the circumoral ring of cilia of various larvæ indicates, as I have already explained, not a widely distributed ring of cilia, but a special tract of ectodermal differentiation, which may give rise to cilia or tentacles or sense organs, or, perhaps, other structures which require special connection with the nervous system. The cilia of the free swimming larvæ are therefore not homologous with the tentacles of Hydra, but the ectodermal differentiated band in the one case producing cilia, is homologous with a similar band in the other case producing tentacles. The simple hydroids are therefore to be looked upon not as simple gastrula, but as

representatives of a slightly later stage; a stage retained temporarily in almost all animals possessing free larvæ, and which we have called the pilidium.

Our next inquiry is to discover how different larvæ are related to the pilidium form, or in other words, to find out, if possible, along what line this pilidium form has been modified to produce the various types of existing larvæ. The first modification to take place in the transformation of the pilidium into other forms is the completion of the digestive tract, the transformation of the gastrula sac into a continuous tract opening by mouth and anus. Wilson, indeed,[1] has shown that the initiatory steps have already been taken toward this direction in certain of the more complex Alcyonaria. The various conflicting evidence on the relation of mouth and anus to the blastopore is too well known to demand repetition. The relation is one that undoubtedly admits of great variation. Of all the suggestions advanced to explain the various differences, by far the most plausible is that of Sedgwick.[2] According to this view the blastopore is the beginning of both mouth and anus. It originally elongated, one end becoming the permanent mouth, the other becoming the anus, while the intermediate portions closed up to form the ventral surface.

This view of Sedgwick's has received some considerable confirmation from the observations I have made on Thalassema and Serpula. I have already in these Studies given a brief account of the formation of mouth and anus in Thalassema. An examination of Pl. III of the present volume of these Studies will show what actually takes place in this animal. Thalassema has been described as having the blastopore converted into the mouth.[3] But these figures will show that it is not strictly true. The only evidence of the blastopore at first is a flattening at the oral pole. As the larva develops, one extremity of this does become the mouth, while the anus is formed at a spot which is really the other extremity of the flattening, though at the time the anus is formed it is at a considerable distance from the mouth, owing to the elongation of the body. This is, of course,

[1] Wilson. Mesenterial filaments of Alcyonana. Naples Mittheil. 1884.

[2] A. Sedgwick. Origin of Metameric Segmentation. Quar. Jour. Mic. Soc. 1884.

[3] Kowalwsky, Zeit. f. Wiss. Zool. xxii, p. 284.

not strictly the conversion of blastopore into mouth and anus, but it is so easily seen to be derived from such a process that it may be taken as a good argument in favor of Sedgwick's view.

A much better piece of evidence is the case of Serpula, which I have had the good fortune to study during the last summer. Here the blastopore has been described as becoming the anus,[1] but a careful study has shown me that a mistake was made here similar to that made on Thalassema. I have in Figs. 11 and 12 endeavored to illustrate what actually takes place. The blastopore is formed in the usual way, and at the time of its appearance there is present the aboral sense cilia and the circumblastoporal ring. The blastopore soon becomes an elongated slit, extending in a direction connecting the future mouth and anus. The opening becomes now closed, and the endoderm remains in connection with the ectoderm by a long area (Fig. 11). The body elongates obliquely, as shown in Fig. 12. The solid gastrula sac now becomes separated from the ectoderm at its middle, remaining in connection with it only at its two extremities. Up to this time it is solid, but now a cavity appears in it, one end becoming open as the mouth, and the other some time later as the anus. In this case, therefore, the blastopore becomes converted directly into mouth and anus. This is the more suggestive since the whole development of Serpula shows every indication of being very primitive, and the occurrence of this history of the blastopore in this form is good evidence of its primitive importance.

I find in the development of Thalassema and Serpula, therefore, evidence for believing that the relation of mouth and anus to the blastopore is somewhat as Sedgwick suggests. And this leads me in the study of larvæ to an important conclusion, as follows: All larvæ which possess in their gastrula stage a circumblastoporal ring must, upon the subsequent completion of the alimentary canal, have both mouth and anus on the same side of this ring. We have seen that the pilidium is divided into a preoral and oral part by this ciliated ring, and now since the blastopore is entirely upon the oral side, it follows that both mouth and anus must be upon the posterior side of the ring, which therefore

[1] Stossich, Sitz. d. K. K. Akad. Wiss. Wien. B. lxxvii, 1878; also Drasche, Z. A. 1883, p. 506.

becomes always a preoral band. This is easily seen from the Figs. 10–14. The mouth and anus, therefore, with the ciliated band become landmarks by which different larvæ can be compared with each other.

The next change necessary in order that our pilidium type should be transformed into any much more complicated form, either as larva or as adult, is obviously that the body becomes elongated. And, indeed, this does take place in every instance. But it is evident from the examination of the pilidium type that this elongation may take place in quite a number of different ways. We can actually trace two different methods of this elongation. First, we find that in one large group of animals, the preoral lobe (anterior to the ciliated ring) elongates to form the body of the animal, while the oral lobe remains relatively very small; or second, we find in another large group the oral lobe elongates to form the body while the preoral lobe remains relatively very small. And these two different types of growth give rise to two entirely different types of larvæ and adults.

The first of the two groups which I will consider is the one in which the elongation of the preoral lobe has taken place. Here belong the Coelenterata, Polyzoa and Brachiopoda.

Coelenterata.

The usual form of Coelenterata larva is the Planula; but the Planula has no mouth, no digestive system, and, indeed, all evidence tends to show that it is not a primitive form, while that of Actinia or an Octocorallum, Fig. 2, is much more so.[1] The relation of the hydroid to this form has already been pointed out. It simply attaches itself by its aboral extremity and elongates. But this elongation, as may be seen by comparing Figs. 2, 5 and 6, is confined mostly to the preoral lobe. The circumblastoporal ring remains always quite close to the mouth, and becoming expanded into tentacles, is converted into the circumoral ring of tentacles. In many Hydrozoa and Actinozoa the adult becomes, of course, much modified, and is therefore more than a stationary pilidium; but these modifications are truly secondary, and do not affect the relation of the animal as a whole.

[1] See discussion upon Invagination and Delamination, by Balfour in his Comp. Emb.

But there is another form among Coelenterates which, in many cases, is the true adult, and which seems at first sight to be built upon a different type. I shall not attempt here to enter into a discussion of the vexed question of the phylogenetic relation of the hydroid and the medusa, beyond expressing myself as essentially in agreement with the views of Bohn[1] and Brooks,[2] to whose papers I would refer. Bohn, in a very interesting paper on the subject, makes use of the remarkable likeness of the larval forms known as Actinula, found in Tubularian hydroids, to both a hydroid polyp and a medusa; and comes to the conclusion that both hydroid and medusa were originally derived from something like the Actinula. Indeed, Actinula is most readily compared with either hydroid or medusa. In Tubularia, where Actinula is the larval form, the adult is derived directly from it, Figs. 5 and 6: while in certain trachymedusæ, *e. g.* Aeginopsis, an actinula form is converted directly into a medusa. Moreover, the remarkable medusa of Clavetella prolifera[3] gives further evidence for believing in a close relationship between Actinula and the medusa. Finally, the remarkable history of Cunina shows in a very interesting manner how a hydroid form may become a medusa. Figs. 3 and 4 were kindly lent me by Dr. Brooks, and will illustrate the point. Fig. 3 may be directly compared with the Actinula of Porypha, Fig. 5, and differs from it only in the long oral lobe. But Cunina larva, Fig. 3, is in the subsequent metamorphosis converted into a medusa, Fig. 4, while Actinula is converted into a stationary hydroid, Fig. 6. The relation of the Actinula to the pilidium form we have already seen, and we have thus linked together the hydroid Medusa, Actinula and pilidium forms.

The relations of the Coelenterata seem to be therefore briefly this. A primitive form, which is essentially a pilidium, becomes modified in two directions. On the one hand it attaches itself by its anterior end, and its preoral lobe elongates, thus forming a hydroid. On the other hand it remains locomotor, but its body elongates transversely, in such a manner as to carry the circumblastoporal nervous ring away from the axis of the body.

[1] Bohn, Heligolander Medusen. Jenanders Zeit. xii.

[2] Brooks, J. H. U. Circular No. 22.

[3] Allman, Gymnoblastic and Tubularian Hydroids, Vol. II.

This elongated portion of the body grows down around the mouth in the shape of a bell, as in Fig. 4. The nervous ring is thus carried down to the margin of the bell and here gives rise to tentacles or sense organs, and thus we have a true medusa. The exumbrella is therefore homologous with the body of the hydroid, while the subumbrella is homologous with the oral disk of the hydroid, and the circumoral tentacles of the latter are homologous with the marginal tentacles of the former.

Polyzoa.

Of the two groups of Polyzoa we need consider only the Entoprocta. Their adult structure shows them to be the simplest forms, they are nearest the embryonic stages, and the Ectoprocta in their development partially pass through an Entoproctous stage, while the others have such a highly complex development as to indicate that they are greatly modified. To the Entoprocta, therefore, must we look for evidence as to the significance of the larvæ.

Studying the development of the best known Entoproctous larva, Pedicellina,[1] we find the early development quite coinciding with that which we have found reason to think is primitive. The gastrula is an invaginate one, and there is soon formed around the blastopore a well-marked ciliated ring, and at the aboral extremity is developed a "ciliated disk," which undoubtedly represents the sensory tuft of cilia which we have seen in Actinia and pilidium. In other words there is formed a true pilidium stage, in all essential respects like that found in Coelenterata.

Now, however, the Polyzoan departs from the line taken by the Coelenterata, in the formation of an anus. And this opening is formed in essentially the same manner as that of Serpula, already described. The blastopore elongates and comes to occupy a position extending between the future mouth and anus, *i. e.* the ventral surface of the animal. One extremity remains open as the mouth, while the rest closes up. But soon the anus appears at the other extremity of the blastopore, and therefore within the ciliated ring, and in a position which corre-

[1] Hatschek, Zeit. f. wiss. Zool. XXIX.

sponds with the Annelid's anus (Fig. 7). Now the preoral lobe elongates somewhat while the oral lobe remains relatively small, in the same manner as in Coelenterata. There is thus produced the larva (Fig. 7) which differs from the pilidium larva and the Coelenterate larva in the single point of possessing an anus.

The relation of this larva to the adult is much the same as that we have traced in the Coelenterata.

The preoral lobe elongates to form the body, and the ciliated ring grows out into a row of tentacles, the Lophophore. Fig. 8 is a figure of Loxosoma, showing plainly its relations to the larva, Fig. 7. Thus, the only difference between the Coelenterate and the Polyzoan is the fact that in the latter group the blastopore becomes closed at its centre, leaving the two extremities open, as mouth and anus, while in the former group the blastopore remains permanently open throughout its whole extent, although, as Wilson has shown,[1] the initiatory steps towards differentiation into mouth and anus may sometimes be taken. It is true that the attachment by the ciliated disk, as above assumed, has never been described, but it is so obviously the simplest method for the formation of the adult that Balfour unhesitatingly came to this conclusion. Moreover, I am informed by Mr. Nachtrieb that in some work he did at Hampton during the summer of 1883, he was fortunate enough to find the young Pedicellina attached by its ciliated disk to a piece of grass. He was unable, however, to raise the animal and study its further development. On the other hand, Barrois[2] has described the process quite differently. According to him, the attachment takes place by the oral extremity, and the subsequent metamorphosis is very complicated. These observations however do not militate against the homology here drawn, but against the view of the simple metamorphosis above

The comparison between these two animals of our first group is quite striking. In both the Coelenterate and Polyzoan we find a pilidium larva; in both, the preoral lobe elongates to form the body of the adult, while the oral lobe remains relatively small. In both, the circumblastoporal nervous ring gives rise to tentacles, and finally it is in these two groups *only* that we meet with

[1] Wilson. Mesenterial filaments of Alcyonana. Naples Mittheil. 1884.
[2] Barrois. Ann. and Mag. Nat. Hist. 1882.

a tendency to form branching columns, so similar as to lead often to the confounding of the two. The only essential difference is the separation of the blastopore into mouth and anus, a process begun in Alcyonaria and completed in Polyzoa.

Brachiopoda.

Closely related to the Polyzoa are the Brachiopoda, whose history must have been much the same. The similarity of the adults has been frequently noticed, and Brooks[1] has shown that the Brachiopod larva may be directly compared with the adult Polyzoan. Brooks' paper and arguments are quite full, and since very little evidence has accumulated since the publication of his paper, it will be only necessary for me to give his conclusions. He decides that both the adult structure and the larval history teach that the Polyzoa are the nearest allies of the Brachiopoda, since the adult Polyzoan is essentially a larval Brachiopod, and since the early larvæ are reducible to the same type (which is essentially our pilidium type). No one since that time, as I am aware, has objected to this conclusion except Balfour. In his Comparative Embryology he admits the plausibility of the view, but raises two objections to it: 1. "The lophophore is pre-oral in Polyzoa and post-oral in Brachiopoda," and 2. "The concave side of the lophophore is turned in nearly opposite directions in the two forms." The only Brachiopod with a free larva which has been studied in its early stages is Argiope, but an examintion of the larva, Fig. 9, will show that Balfour's objections are not well taken. His understanding of the Brachiopod larva was wrong, and he confounded the anterior with the posterior end. A glance at Fig. 9, and the consideration that the preoral lobe has elongated, together with the fact that the mouth subsequently appears at the lower side of the figure, will show that the extremity which Balfour called posterior is really anterior, while his cephalic lobe is really the oral lobe of the pilidium and Polyzoa. The row of tentacles developing from the ciliated ring is, therefore, anterior to the mouth in either case. The second objection is now of no moment, since we have seen that the ventral and dorsal surfaces are not differentiated in these

[1] Lingula. In these Studies, Vol. I.

forms. We may safely assume, therefore, that the study of the adult and the larval history indicate that Brachiopoda and Polyzoa are related to the pilidium form by being attached by the anterior end, and having their preoral lobe elongated. The observations of Barrois, if confirmed, may cause us to modify the view as to the attachment of the larvæ, but not as to their homologies.

The position of the anus in the Ectoproctous polyzoa and the unarticulated Brachiopoda presents a little difficulty. In these two forms the anus is situated outside the lophophore in positions that cannot be homologous to that of the Ectoproctous forms. To explain this it is necessary to assume either that the anus in these cases is a new formation or has at least changed its position, or that the lophophore has moved. If there were no reasons to the contrary the latter assumption would be the more natural. But when we come to consider, (1) That this Ectoproctous anus is somewhat variable, (2) that it is entirely lost in a majority of Brachiopods, (3) that it has an entirely different origin from that of the Entoproctous Polyzoa, arising as a separate opening, while the anus of the Entoprocta is a part of the blastopore: (4) that some Ectoprocta pass through an Entoproctous stage (Cyphonantes), and (5) finally, that if this Ectoproctous anus be considered as the same as the Entoproctous anus, the lophophore must be considered to pass through the blastopore: I think it is abundantly evident that the latter of the two assumptions is the more probable. With this assumption there is no longer any difficulty. The relations of the members of the group would be something as follows: The earliest form is the Entoproctous Polyzoa, in which the blastopore became elongated and differentiated into mouth and anus within the circumblastoporal ring. From this form are derived the Ectoproctous Polyzoa, either by the formation of a new anus, or by the old anus being moved outside the lophophore. To this latter group finally are related the Brachiopoda, which in the more primitive forms retain the Ectoproctous anus, and in the later more modified types have lost it altogether.

The study of the larvæ and adult forms of Coelenterata, Polyzoa and Brachiopoda leads us thus to the conclusion that they form a logical group by themselves, which have been

derived from the pilidium type by the elongation of the preoral lobe, and the final attachment of the animal by its anterior end. The Coelenterata, on the one hand, have blastopore undifferentiated into mouth and anus; and on the other hand, the single group consisting of Polyzoa and their near relatives Brachiopoda have mouth and anus.

On the other hand, the pilidium larva in becoming modified to form the adult has its oral lobe elongated, giving rise to a second logical group of which the Annelids and Mollusks are the best examples, but which also probably contains the Sipunculids and Planarians.

Annelids.

In a large majority of Annelids the early history is very much disguised by the presence of a large amount of food yolk, so much so that it is impossible to make out any logical history. An irregular segmentation, an epibolic gastrula, a ciliated larva with no mouth, Fig. 15, is the usual history, and is one which is undoubtedly in nearly every step highly modified. But a few forms have been studied which present us with a more primitive history. Polygordius, Thalassema and Serpula are such examples. From these forms with little food yolk and primitive history a pretty complete series of gradations can be found, in which the food yolk is becoming more abundant, and the early stages more and more unlike those of Serpula, etc. In all of the forms there is eventually an approximation to the Serpula form, and the more abundant the food yolk the later does this approximation occur. In Arinicola, of which Fig. 15 is the young larva, it occurs very late, hardly before the animal assumes the adult form; while in the Annelid of which Fig. 17 is a representation, the approximation occurs much earlier; and this is due, largely at least, to the presence or absence of food yolk.

As a type of the Annelid history I will take Serpula. Serpula possesses first a typical pilidium stage, Fig. 10. The method of the formation of anus and mouth, Figs. 10–13, has been already described; while those changes are taking place the body elongates. This elongation is not in the line of the gastrula axis, but somewhat obliquely to it, and concerns almost entirely the oral lobe. By the elongation the anus, which was originally part of the blastopore, is carried farther and farther away from

the mouth, the region of the closure of the blastopore lips elongating to form the ventral surface, Figs. 11–13. Meantime the preoral lobe retains its original size or grows but slightly, and finally, as is shown in Figs. 13 and 14, becomes the cephalic segment of the adult Annelid, or the so-called proboscis of the Echiuridæ, Fig. 16. This history, which is essentially the same for Serpula, Polygordius and Thalassema, may be considered as typical, and shows us that it is the oral lobe which elongates, in contradistinction to the Coelenterate group.

This understanding of the Annelid enables us to draw an instructive comparison between the Coelenterata and Polyzoa on the one hand, and the Annelid on the other. We see first that the cephalic segment of the Annelid (the preoral lobe or proboscis of Thalassema), which Hatscheck has shown not to be equivalent to the other segments,[1] is really the representative of the whole of the body of the Coelenterata and Polyzoa outside of the row of tentacles. This region is uppermost in all of my figures. The body of the Annelid is equivalent to that part of the Coelenterate behind the tentacles, or in the Polyzoan that part of the body inside the lophophore. More particularly the ventral surface of the Annelid's body is unrepresented in Coelenterata, since it is formed by the closure of the lips of the blastopore, and in the Coelenterata the blastopore is not closed. In the Polyzoa, however, the true ventral surface is present. The region between the mouth and the anus, which Hatscheck has shown to be formed by a closure of the lips of the blastopore, is the true ventral surface (marked V in the figures). The dorsal surface of the Annelid if we trace it back from Fig. 14 to Fig. 10, is seen to be represented in the pilidium larva by the small space between one end of the blastopore and the circumblastoporal ring. This region is, of course, present in the Coelenterata and Polyzoan (marked D in my figures). Finally, this view will imply that the circumblastoporal ring of differentiated ectoderm has given rise, in Coelenterata, to the tentacles of the polyp form and the marginal nerve bodies of the medusa; in Polyzoa to the lophophore; and in Annelids it has frequently disappeared, since its function as nervous structure has been supplied by the newly developed ventral nervous system. This band has not always

[1] Hatscheck. Studien u. Ent. d. Anneliden. Arb. a. d. Zool. Inst. Wien, I.

disappeared, even in Annelids, but is frequently retained in the shape of the tentacles, which are developed in front of the mouth in a position homologous with the lost ciliated ring.

The homologies above given may seem rather strange, but a slight consideration will show that they are the outcome of the simple understanding that it is the oral lobe that has elongated in one case, and the preoral lobe in the other. And, further, it will be seen that they are in exact agreement with the views of Sedgwick and Wilson. I wish to say, however, that my own conclusions upon this subject were formed independently of these two papers. In a note published two years ago,[1] I indicated that my work on Thalassema had led me to such views. I simply refer to this to show that my conclusions are not copied from those of Sedgwick, but were derived from an entirely independent source of evidence.

Mollusks.

In many respects Mollusks agree in their development with Annelids. In considering this type we must bear in mind that in a majority of cases the presence of a large amount of food yolk has greatly modified the history. No one will pretend to claim that the development of Cephalopoda can be in any sense primitive. Nor, indeed, can that of the ordinary gastropod be considered much more so; as, for instance, that of Nassa. We cannot expect, therefore, to get much aid from such forms, but must look for cases where the food yolk is relatively small in amount. A number of such instances can be found, but perhaps the best for our purpose are Paludina,[2] and Teredo.[3]

That Molluska do possess the evidences of a pilidium stage is shown by Fig. 18. This figure, taken from Blütchli's work on Paludina, shows a pilidium with the exception of the absence of the aboral sensory plate. But whether it be present here or not is not of much importance, since it undoubtedly is present in many Mollusks (Cardium, Teredo), and we thus see that a true pilidium is here found.

[1] J. H. U. Circular No. 22.

[2] Lankester, Quar. J. Mic. Sci. XVI, and Blütchli, Zeit. f. Wiss. Zool. XXIX.

[3] Hatscheck u. Entwick. von Teredo, Arb. a. d. Inst. Wien. III.

Starting with such a form we find the larva which results from it to be quite different in different cases. Teredo will be particularly instructive, since the full-grown larva is so similar to the ordinary Annelid larva. Fig. 20 is the larva of Teredo, and it is a good trochosphere with a very striking likeness to that of Serpula. Its early history is quite different, but the resulting larva is almost the same as that of any Annelid. This likeness was readily seen by Hatscheck, and he based some important deductions thereon. He even went so far as to predict from his researches on Teredo that it would eventually be found that the intestine of Annelids was endodermal and not ectodermal, as had been previously stated—a prediction which I have had the good fortune to verify.

The larva of Teredo then is a typical trochosphere, arising from the pilidium in essentially the same manner as that of the Annelid. One thing is important to notice. The preoral band of cilia becomes the velum of the young Mollusk. But even more conclusive on this point is the history of Paludina. In this form the circumblastoporal ring, Fig. 18, becomes the velum, Fig. 19, showing conclusively the relation of the velum to the preoral ciliated band of Annelids, etc. As the Paludina larva develops, the oral lobe elongates very much, the preoral lobe being left as a very small tract within the velum. In this animal the blastopore becomes the anus, according to both Lankester and Blütchli, a point which, however, presents us no difficulty, when we consider that both anus and mouth are derived from this opening. The mouth when it appears, Fig. 19, is posterior to the ciliated band, as we should expect, and the result is again a trochosphere larva differing considerably from Teredo, but with the same fundamental parts, and evidently arising in the same way by the elongation of the oral lobe.

Or again, examine the development of Cardium[1] or the oyster,[2] two forms with not a great amount of food yolk, and we always find a larva which is essentially a trochosphere, with the mouth just behind the velum, and the anus posterior.

The velum is seen in these cases to be homologous with the preoral ring of Annelids, and these instances given are enough to

[1] Loven. Vetnsk. Akad. Handl. 1848. [2] Brooks. In these Studies, Vol. I.

demonstrate this homology. Frequently the velum is not a complete ring, being opened behind, but the same is true of the preoral ring of Annelid larvæ (Polygordius), and even in pilidium, occasionally the circumblastoporal ring is not complete. In Mollusks the velum, usually in the later larval stages, grows out into large ciliated lobes which are capable of contraction, but this is of course a secondary affair, and may be compared to the expansion of the same band into tentacles in the Coelenterata.

Assuming that the trochosphere larva is the typical form for the Mollusks, it becomes at once evident that the Mollusks and Annelids are closely related, being derived from the pilidium form in essentially the same way. In both cases did the oral lobe elongate at the expense of the preoral lobe, and in both cases did this elongation take place, not in the line of the gastrula axis, but obliquely to it,[1] in such a manner as to leave the mouth near the circumblastoporal ring, while the anus was carried backwards to the extreme posterior end. In the Mollusk the preoral lobe is even smaller than in the Annelid, but it usually carries the typical bunch of sensory cilia.

We can now easily compare the larva with the adult. Various comparisons of the Mollusks and the Annelids have been made, but an examination of the figures of Plate IX leaves but one possibility. The body in both cases is the highly developed oral lobe of the trochosphere. The shell of the Mollusk, Fig. 20, arises on the surface of the body equivalent to the dorsal surface of the Annelids. The foot of the Mollusk is the ventral surface of the Annelids. The velum is simply a larval organ, but the part of the body within its area is equivalent to the cephalic lobe of the Annelids or the proboscis of Thalassema, Fig. 16. In both Mollusks and Annelids, therefore, the oral lobe elongates at the expense of the preoral lobe, but the resulting body in one case covers itself with a shell, and in the other becomes segmented.

There now remains for consideration the larval forms found among Rotifera and Planarians, the two peculiar larvæ Actinotrocha and Tornaria, and the larvæ of Echinoderms.

The Rotifers may be easily disposed of, since the adult Rotifer is nothing more than a trochosphere. It is, therefore, undoubtedly to be classed with Annelids and Mollusks.

[1] See Hatschek.

Planarians have been comparatively little studied, and the formation of their young larva is not much known. I shall therefore not attempt to discuss them. The figures of Lang[1] suggest to me that the so-called post-oral ciliated band of the Plananaus larva is, in reality, a preoral band which has become quite altered in its position by growth. I have not yet seen the text which describes these figures, and cannot therefore draw any conclusions from them.

Actinotrocha.

The larvæ of Phoronis and the Sipunculids are in many respects peculiar. I have had no opportunity to study them, but their history, as described by Hatscheck and Caldwell, shows them to be by no means closely related to the other so-called Gephyreans. Caldwell[2] believes them to be somewhat closely related to Brachiopoda, but it is hardly possible to judge of the truth of this conclusion until the publication of Caldwell's complete paper, and the clearing up of some of the obscure problems.

Tornaria, the larva of Balannoglossus, has been considered to be an Echinoderm larva, and its similarity to these larvæ is so great in the ciliated bands, water system, etc., as lead without doubt to their classification in the same group.

Echinodermata.

When we come to study Echinoderm larvæ we immediately meet with differences which lead to the conclusion that we have in the animals here related, a third group radically set off from the other two. We first meet with a difficulty from the fact that there is such a complicated metamorphosis, and that the larva is never, with the exception of Synapta, transformed directly into the adult. This exceptional history of Synapta is one good reason for believing that Synapta stands nearer to the primitive Echinoderm type than any of the other groups. But, ordinarily, so complicated is the metamorphosis which the larvæ go through, that no conclusions from the larvæ can be drawn as to the relations of the adults. Having recognized this

[1] Lang. Naples, Fauna and Flora, Polydaden.

[2] Caldwell. Anatomy and Development of Phoronis, Proc. Roy. Soc. Vol. XXXIV. Abstract.

fact, that the larvæ are undoubtedly very highly modified, we are prepared to meet with difficulty when we try to find their relations to the other types we have studied.

And when we come to try to recognize a pilidium stage in Echinoderms we meet with nothing which is exactly a pilidium. In the first place, we find that the blastopore always becomes the anus and never the mouth, except in those forms which possess no anus. This difficulty would not be a very great one if it stood alone, for reasons we have above seen, but since it is connected with other greater differences it is probable that it has also its significance.

The stage of Echinoderm larvæ which corresponds to the pilidium I have not figured, since Mr. Nachtrieb, in a paper in preparation, has very carefully figured and described it. In brief it is this, a typical gastrula, with a mouth at one extremity, at the other a bunch of long cilia. The whole gastrula is covered with tolerably long cilia, but at the aboral pole there is a long tuft, accompanied by an ectodermal thickening. It is also interesting from the fact, that with the subsequent growth of the larva they become changed in position, and finally in the full grown larva can no longer be seen, which is a further evidence of the great modification of the Echinoderm larva.

Beyond this the resemblance to the pilidium does not go; for there is found nothing to correspond to the circumblastoporal ciliated ring. All of the other larvæ which we have examined have a ciliated band around the blastopore, and both mouth and anus when formed are upon the same side of this band, which is consequently both pre-oral and pre-anal. No such a band is found in Echinoderms at any period. With the growth of the larvæ various ciliated bands make their appearance, and one of them in Holothuroids is a circumoral band. Gegenbaur has ingeniously shown (see Fig. 57 of his Comparative Anatomy) how such a band might be converted into a pre-oral and post-oral band such as are present in the Asteroid larva. He may be right for the cases in question, but when he homologizes them with the ciliated bands of similar names in the trochosphere, he has gone beyond legitimate conclusions. In the first place there is nothing in the development of these bands to indicate such a

conclusion. Even in Echinoderms the circumoral band arises from the fusion of a number of others, usually four, and never do we find a single band dividing into two. In the Annelid trochosphere we have seen that the preoral band is always a complete ring derived from a circumblastoporal ring of the pilidium stage. This ring is therefore undoubtedly a distinct one, and not derived from the division of a previously existing circum-*oral* ring. Nor can this whole circumoral ring be considered as homologous with the ring of the pilidium, since that is circum-*blastoporal*, while this Echinoderm band is strictly circum-*oral*. In short, there is nothing as yet found in Echinoderms which can correspond to the circumblastoporal ring of the pilidium and the preoral ring of the trochosphere.

What, then, is the relation of the Echinoderm larva? Balfour[1] was inclined to believe that we have here a remnant of a form even earlier than the pilidium form, and therefore an earlier branch. I am inclined, however, to the opposite conclusion, and consider it as a much later form in which the pilidium characters have been partly lost. I have three chief reasons for this conclusion:

In the first place, we have just seen that while the Echinoderms do not pass through a true pilidium stage, they do have differentiated the anterior nervous tract. This is afterwards lost, but its presence in the young larva is a sure indication of relationship to the forms we have already studied.

Secondly, the presence in Echinoderms of a mesoderm indicates the same thing. Echinoderms always possess a mesoderm quite similar to that of Mollusks. Such a mesoderm is not present in Coelenterata, and since Coelenterata do have a true pilidium stage, the inference is that Echinoderms have also once possessed this stage and are of later rather than earlier origin.

Finally, it seems to be the tendency of modern research to unite into one large group the Echinoderms, Nemertians, Balannoglossus and the Vertebrates, as quite radically set off from the rest of the animal kingdom. The likeness of Tornaria and Echinoderm larva is very striking in every respect. The work of Hubrecht shows that Nemertians and Vertebrates are related, and finally, Balannoglossus shows many resemblances to the

[1] Comp. Emb. Vol. I.

Nemertians. Now Nemertians pass through a pilidium stage; indeed, the term has been borrowed from the name of their larva. This being the case, one of three alternatives is necessary. Either (1) the pilidium larva has no significance, or (2) the Nemertians have no relationships either to Echinoderms or Balannoglossus, or (3) Echinoderms also belong to the same group with the pilidium larva. I think the universality of the pilidium form is a sure indication that it is a highly significant stage. The work of Balfour, Hubrecht and Bateson, together with the likeness in the adult anatomy of Nemertians and Balannoglossus, induce me to accept a relationship between them. And I therefore prefer to accept the third of these alternatives and consider that the Echinoderms are also related to the pilidium form.

But if this be true, then the ordinary Echinoderm larva is a highly modified larval form, derived from the pilidium by a complicated method of growth, so much so that the relation of the adult to the larva is entirely secondary. What may be the primitive relation of the Echinoderm I will not attempt at present to say. A study of the development of Synapta for the purpose of deciding the question would doubtless lead to valuable results. The last work on this subject, that of Salenka,[1] seems to indicate that the gastrula is elongated something as is that of the Annelid above described.

The general result of this paper is therefore briefly as follows. All animals which possess free larval forms, leaving aside the Arthropods, the Sponges, and the parasitic forms with highly modified history, can be related to each other through a form which is essentially like a pilidium, consisting of a gastrula with a preoral tuft of cilia and a circumblastoporal band, both tracts indicating probably nervous differentiation. From this form the various larvæ and adults have been so derived as to fall into three widely separate groups. The first group was divided by the elongation of the preoral lobe, by the expansion of the ciliated band into tentacles, and usually by the attachment of the animal by its anterior extremity. In this group are found the Coelenterata, with blastopore undifferentiated, the Polyzoa with blastopore differentiated by elongation and closure at its centre into mouth

[1] Salenka. Studien u. Ent. des Thiere. Wiesbaden, 1883.

and anus, and the Brachiopoda derived from a modified form of Polyzoa. In the second group the oral lobe elongated, the blastopore differentiated into mouth and anus, the animal remains free, and the circumblastoporal ring frequently disappears in the adult, though sometimes indicated by tentacles. Here are found the Annelids, the Mollusks, and probably also the Planarians. A third group contains Echinoderms, Balannoglossus, and probably the Nemertians and Vertebrates, whose relation to the pilidium is not yet clear, but which are probably derived by elongation of the oral lobe. In this group the larvæ are the most highly modified, so that the full grown larvæ gave no indications of the relations of the adults, nor of the relation of these larvæ to the early pilidium type, although the early stages retain undoubted traces of the pilidium ancestry.

EXPLANATION OF PLATES.

Plate VIII. Forms resulting from Elongation of the Preoral Lobe.

Fig. 1. Pilidium type.

Fig. 2. Unknown Actinozoan.

Figs. 3 and 4. Two stages in larva of Cunina, showing method of formation of medusa from one primitive form.

Figs. 5 and 6. Porypha, showing formation of hydroid form.

Figs. 7 and 8. Polyzoa. Fig. 7 is early larva of Pedicellina. Fig. 8 is a later stage of Loxosoma.

Fig. 9. Argiope.

Plate IX. Forms resulting from Elongation of the Oral Lobe.

Figs. 10–14. Various stages in the development of Serpula.

Fig. 15. Young egg embryo of Arenicola.

Fig. 16. Late stage of Thalassema millita.

Fig. 17. An unknown Annelid larva.

Figs. 18–19. Two stages of Paludina.

Fig. 20. Trochosphere of Teredo.

Figs. 7 and 20 after Hatscheck. Figs. 3 and 4 from Brooks. Figs. 1 and 9 from Balfour. Figs. 18 and 19 from Blütchli.

OBSERVATIONS ON SEVERAL ZOOGLOEAE AND RELATED FORMS. By WILLIAM TRELEASE, Sc. D.[1]

While in Baltimore, in January, 1884, I was kindly given specimens of several pigment bacteria by Mr. A. L. Webster, Fellow of the Johns Hopkins University. These belonged to the species described in the following pages as *Bacterium aurantiacum*, *B. luteum*, *B. incarnatum* and *B. candidum*. On my return to Madison, in February, cultures were at once started, with a view to obtaining material for class use; but before they had been continued long, several other interesting forms appeared, so that a series of systematic inoculations was undertaken, with the result of showing that the several species under observation, though often scarcely distinguishable under the microscope, reproduce themselves truly, perpetuating certain macroscopic characters manifested in the color and form of their zoogloea-masses, by which they are as certainly distinguishable as species of the higher plants. Although readily recognized, however, these species could not be identified with the chromogene forms recognized by writers on bacteria, because of insufficient descriptions and the dearth of authentic specimens; and it is not without hesitation that I venture to bestow names upon them, although it is hoped that the results of this study may contribute to their subsequent recognition, whatever names they may ultimately bear.

During the course of my cultures I was favored with one or two other forms, notably that described under the name of *Bacterium violaceum*, by Professor W. G. Farlow, of Harvard University, to whom I am also indebted for the *exsiccatae* referred to under several species. When the work was begun I was under the impression that all of the forms were introduced;

[1] The following resumé of some observations made in my laboratory during the winter and spring of 1883–4 is, in substance, the same as a thesis in Natural History offered to the Academic Council of Harvard University in 1884.

but as it continued, new forms appeared in sufficient quantity for isolation, and the fact that they are truly indigenous was demonstrated by subsequent cultures undertaken the following summer at the Botanic Garden, in Cambridge, Mass., the starting point of which was the germs obtained by rubbing slices of boiled potato on the floor, in a sink, and in similar places, where organic dust could most easily collect. These, when cultivated in a building where pigment bacteria have not been kept, gave me nearly all of the forms previously studied, as well as one or two others, not isolated in my earlier cultures.

At first various starchy substances were employed as media for the growth of the different species, but I finally restricted my cultures to boiled potatoes carefully cut in halves, the exposed face being inoculated in one or more points by means of a needle scrupulously fired before and after each inoculation. These were kept in sauce-plates, under inverted tumblers, some of which were not disturbed until the end of the culture, while others were opened from time to time for microscopical study or for material to be used in starting new cultures. It is needless to say that many of these failed through the introduction of other germs in one way or another; but by means of frequent transfers, pure cultures of all ages were kept under daily observation for about three months. It would have been both interesting and instructive to cultivate the species in nutrient gelatine, agar-agar, etc., but for various reasons such cultures were not undertaken. Fluid cultures, in a study of this kind, are of little value.

Each species was studied in two respects. The texture of the zoogloea, and its general form, color and surface markings were subjected to frequent examination; while the microscopic characters of its component cells were made out under a sufficient enlargement by a study of fresh material and of slides stained with methyl-violet and prepared according to the well-known method of Koch. Unlike Rasmussen,[1] I have not noticed that this preparation appreciably changes their form or size, and it has been constantly employed, all of the figures being drawn from freshly prepared methyl-violet slides. These figures, which represent very nearly the range of variation in the cells, were

[1] *Cf.* Poulsen: Bot. Centralblatt, XVII, No. 12, p. 390.

drawn with the camera, and are uniformly enlarged 2500 diameters unless otherwise marked. This was done with a Leitz $\frac{1}{16}$ homogeneous immersion and V eye-piece, illumination being afforded by the Abbé condenser.

A number of non-pigment species, and of other interesting bacteria and yeasts, have been introduced at the end of the paper.

1. Bacteria.

Micrococcus, sp. (Fig. 13).

The material received from Mr. Webster gave rise, in a short time, to a red form, which was afterwards sent me by Dr. Farlow. This was supposed to be the well-known *Micrococcus prodigiosus* (Ehr.), which not infrequently appears upon various food substances in Europe; but it was so badly intermixed with *Bacterium candidum* and several Mucors and other molds that few transfers were made, as it passes through an incubation period of several days, during which the accompanying species invariably overran the culture. Where it succeeded in establishing itself it formed small spots of a characteristic color, which was always some shade of magenta. The blood-red and orange tints which *M. prodigiosus* assumes under certain conditions of the culture medium were never produced, but as the microscopic characters of our plant are essentially the same as those of the European, it is doubtfully identified with the latter.[1] The zoogloeae consisted uniformly of nearly spherical cells, .3–.5 μ in diameter (Fig. 13). It was also obtained in cultures of *M. candidus* at the Cambridge Garden, in 1883, where it formed flesh-colored or pink dots otherwise similar to those of the latter species, but with uniformly smaller cells.[2]

Micrococcus candidus Cohn.

Among the first species to attract my attention in the cultures at the Botanic Garden was one which appeared at first in the

[1] For the more important papers concerning *M. prodigiosus* and for further references, see Fresenius: Beiträge zur Mykologie, p. 78–80; Schroeter: Cohn's Beiträge zur Biologie der Pflanzen, I, Heft 2, p. 109–118; Cohn: *op. cit.*, p. 153; and Wernich: Cohn's Beiträge, III, p. 105–118.

[2] Presumably this is the form referred to as *M. prodigiosus* by Bessey (Bull. Iowa Agl. Coll., Nov. 1884, p. 135).

form of small milky dots, scarcely visible to the naked eye, but which soon increased to about 1 mm. in diameter, and became confluent into mammillated masses that ultimately covered the potato, when other species were excluded. Though at first moist, these soon became dry, presenting a dull chalky appearance. This form was reproduced through a number of transfer cultures made by Miss Isabel Mulford and myself, maintaining the same characters.

The zoogloeae agree essentially with those described by Cohn under the above name,[1] both in their macroscopic and microscopic characters. To the naked eye, or under a hand lens, they are very similar to the masses of cells of *Saccharomyces glutinis*, var. *candidus;* their cells are almost perfectly spherical, and .7–.9 μ in diameter.

Fresenius[2] speaks of a white zoogloea occurring on potato, which was, perhaps, this species, since Wernich[3] mentions it as one of those which commonly occur on potato cultures in Europe, though it is far less frequent with us than several others, notably the next.

Bacterium candidum Trelease (Fig. 1).

The species which has provisionally been given this name is by far the commonest of those which I have obtained in the method mentioned on page 194. On boiled or steamed potato, in a warm place, it appears within 24 hours of the beginning of the culture as a white or opalescent zoogloea.

When conditions are very favorable, and especially when the potato is saturated with water, growth is rapid, and it spreads as a thin, smooth, opalescent layer, resembling the potato somewhat in color, though of a very different texture. At first it is moist and glistening; as it grows older the moisture disappears, leaving a dull surface, which, with increasing age or under the influence of drier air, gradually becomes rough, appearing as if sprinkled with a snow-white powder. Soon after this mealy appearance is observable the zoogloea increases considerably in

[1] Cohn: Beiträge zur Biologie der Pflanzen, I, Heft 2, p. 160.
[2] Fresenius: Beiträge zur Mykologie, Heft 3, p. 78, note, and p. 80.
[3] Wernich: Cohn's Beiträge, III, p. 112.

thickness and begins to wrinkle, the folds finally becoming very prominent. Meantime the snowy appearance gives place to a creamy or dull yellow color, which characterizes the old zoogloea.

These stages may all be seen simultaneously on a large specimen. The mealiness is due to a fine lobation of the surface, the several points appearing under a low power much like the glistening grains of finely crystallized sugar, but a little more rounded.

The texture of the mass changes greatly during its development. At first watery or but slightly glairy, it soon becomes ropy and exceedingly viscid, so that in removing portions for inoculation I have several times drawn it into delicate threads upward of half a meter in length, extending from the old zoogloea to the point of my needle. This substance forms a very adhesive paste.

When kept moist and warm for a week or two, molds being excluded, the zoogloea again becomes smooth and glistening, this appearance being sometimes confined to a limited area, but often extending over the entire mass, which, without losing its tenacity, grows somewhat deliquescent, its color remaining nearly unchanged.

In all of these stages the zoogloea consists of a gelatinous ground-substance, in which numerous cells are imbedded. These are quite uniform in diameter, measuring .6–.8 μ. Seen from the end they are round (Fig. 1 *b*) and might easily be mistaken for sphaerobacteria; but from the side they appear ellipsoidal or oblong, and vary in length from 1.6–7 μ, though in old, deliquescent zoogloeae they may not exceed 1 μ. When in active growth they occur singly or, more frequently, in chains of from two to six or seven (Fig. 1). When placed in water the isolated cells as well as the chains often move actively, apparently by the aid of flagella, though these have not been demonstrated. Frequently the long rod-like chains are bent at the points of union of the cells. These zig-zag aggregations commonly rotate as they move forward, when their motion appears to be undulatory.

So far as can be made out, the cells consist of a very delicate wall (aside from the common embedding mass), with nearly

homogeneous protoplasmic contents. The longer rods do not always show segmentation, though they are seldom unicellular. In some cases staining shows that the protoplasm is collected at either end of the cell, the two incipient spores (?) remaining connected by a thin parietal layer of the same substance (Fig. 1 *a*).

This is one of the most persistent of the bacteria which occur on potato. After the first cultures it promptly appeared and developed luxuriantly on every piece of cooked potato exposed in the laboratory, and intruded into other cultures unless the greatest precautions were taken to exclude it; and it has been present in all of the incipient cultures undertaken to obtain new forms, both in Madison and Cambridge. Through a hundred culture generations or more it has maintained its characters unchanged on potato, rice, bread and other starchy substances, and can always be recognized with certainty by the naked eye. On a piece of boiled red beet the zoogloea was of a deep red color, from its saturation with the colored juices, and remained moist for an unusual length of time, drying and becoming wrinkled and mealy only on the margin; but new cultures on potato, started from this, showed only the ordinary form. The species usually passes through its development—exclusive of deliquescence—in from three to five days.

It has been stated that Fresenius refers to a white zoogloea on potato; and at present it seems to be impossible to decide which species he had under observation, though I incline to the opinion that it was *Micrococcus candidus*. Zopf[1] describes and figures a species under the name of *Bacterium tumescens* which is said to be obtained with certainty by maintaining slices of cooked carrot at the ordinary temperature of a room, care being taken not to have them too moist. This species, however, appears from the description given by its author to be distinct from that under consideration, which it resembles more nearly than any other described species with which I am acquainted.[2]

[1] Zopf: Die Spaltpilze, Breslau, 1883, p. 66.

[2] According to Hueppe (Mittheil. a. d. Kaiserl. Gesundheitsamte, II; Abst. in Zeitschr. f. wiss. Mikroskopie, II, p. 110–112), the lactic acid ferment of milk appears on solid culture media in the form of glistening white dots, consisting of cells .3–.4 × 1–1.7 μ, hence smaller than those of this species. The characteristic surface of the two forms is also very different.

Bacterium tumescens Zopf.

The species referred to under *B. candidum*, as occurring in Germany, was obtained in one of my first trial cultures at the Botanic Garden. Appearing at first as semi-fluid dots, less opalescent than those of the preceding species, it soon assumed the definite wrinkled form figured by Zopf, in which it was easily distinguishable from the other by its more yellow color and by the entire absence of mealiness. Though efforts were made to keep it, it was carried through but a few transfers, in all of which, however, it maintained the characters first noted. Its cells are considerably larger than those of the last species, measuring 1.25–1.75 $\times$ 3–6 μ, so that they are morphologically very similar to those of *Bacillus ruber* mentioned later. In the fresh zoogloea they are always arranged in zig-zag chains.

Bacterium aurantiacum Trelease (Fig. 8).

This species gives rise to small zoogloeae of considerable size in two or three days after inoculation, at the ordinary temperature of the laboratory. Like those of other species these vary in size, color and consistence, according to their age and the nature of the potato on which they are grown. When this is watery, and growth is rapid, the zoogloea spreads as a semi-fluid film, of a pale yellow color, approaching orange only in the drier parts; but as it becomes older, and the excess of moisture passes off, it grows waxy, and assumes a persistent orange or deep pumpkin yellow color. Although of slower growth than *B. candidum*, it ultimately covers the potato when other species are excluded. In thickness the mass seldom exceeds .5 mm., and has never been seen to exceed 1 mm. Its surface does not become at all wrinkled, usually remaining smooth or with a slight mammillation.

The structure of these orange zoogloeae is essentially similar to that of *B. candidum*, and their cells, which are sometimes single, sometimes aggregated in chains of several articles, likewise have the power of locomotion when placed in water. In form they are ellipsoidal or oblong, the longer rods often being constricted at points where they are not obviously septate until stained. Their diameter is appreciably smaller than that of the

other species of the genus already referred to (Fig. 8), usually measuring .4–.5 μ—less commonly reaching .7 μ. They are also rather shorter, as the length of representative cells varies between 1.2 and 2 μ. In older zoogloeae their protoplasm generally collects at the ends of the cells, as is shown especially well after they are stained. Central spores are also not infrequent (Fig. 8 *d*).

Beside the distinctions already mentioned this species also differs in a notable manner from most of the others studied, in that it is colored much less readily by methyl-violet, so that a more concentrated solution or a longer exposure to its action is required to stain its cells to the same degree.

In speaking of *Micrococcus prodigiosus* Fresenius[1] mentions orange spots on cooked potato, which consisted of cells resembling those of that species, but slightly larger and more sharply outlined. A name was not given to this form, however, nor was it further characterized.

Twenty years later, in his paper on bacterian pigments,[2] Schroeter refers to apparently the same thing, which appeared, at first, as little spheres of the size of pin-heads, but spread considerably as they grew old. These were composed of micrococcoid cells, devoid of the power of locomotion, from which circumstance, and its color, the species was called *Bacteridium aurantiacum.*[3]

Cohn describes the same form as it appeared and was cultivated in his laboratory.[4] In accordance with his system of classification the species was transferred to the genus *Micrococcus*, still bearing the specific name given it by Schroeter. The cells are described as occurring singly, in pairs or in chains of three or more, and have an oval outline, measuring 1.5 μ.

Seven years later, Wernich found the species in the same laboratory, in his cultures of *M. prodigiosus*;[5] and it seems to be of not infrequent occurrence in Europe, as a beautiful slide of it has been recently sent out by Fischer (Fig. 10), and what appears

[1] Fresenius: Beiträge zur Mykologie, Heft 2 (1852), p. 80.
[2] Schroeter: Cohn's Beiträge, I, Heft 2, p. 119. [3] *l. c.*, p. 126.
[4] Cohn: Beiträge zur Biologie der Pflanzen, I, Heft 2, pp. 189, 154.
[5] Wernich: Cohn's Beiträge, III, p. 118.

to be the same thing was distributed by Von Thuemen,[1] under Cohn's name.

These specimens are all described as being sphaero-bacteria, as I have found the last two to be, by examination; and while the pigments formed are similar, the European species and that which I have grown are evidently very distinct in the character of their cells. As the American form has produced only elongated cells through many cultures, it is improbable that it will ever be shown to have a genetic connection with the similarly named *Micrococcus*, which Zopf[2] includes among those species of which only cocci are known.

Bacterium luteum Trelease (Fig. 2).

Next to *B. candidum* this is the most readily obtained of all the species studied, and may be started at any time by rubbing slices of potato on a dusty floor.[3] Its growth is usually rapid, so that within 40 hours of the time of starting a culture a considerable surface of the potato is covered by the young zoogloea.

On boiled or steamed tubers this appears at first almost fluid, and is nearly colorless; but after growing for a short time, especially if the air is moderately dry, it becomes denser and waxy, slightly more coherent than *B. aurantiacum*, but without the viscidity and ropiness of *B. candidum*. This condition is assumed more promptly on baked potatoes, owing to their drier substance.

After this, the zoogloea, now 1–3 mm. thick, becomes deeply and finely wrinkled, the ridges forming a network .1–.5 mm. high, with meshes of about the same width. Occasionally the surface is tenacious enough to be raised in this process, when it remains for some time as a thin film, stretched from one ridge to another, so that the mass may appear frothy.

It will be seen that although wrinkled, this species differs much in appearance from *B. candidum*, the different character

[1] Von Thuemen: Mycotheca Universalis, No. 1700. "Austria inf.: Wien, in ovo gallinaceo, interdum socia *Bacterii termo* Ehrbg., Sept. 1879."

[2] Zopf: Die Spaltpilze, p. 95.

[3] I suspect that it is this which Bessey (Bull. Iowa Agl. Coll., Nov. 1884, p. 135) refers to *Micrococcus luteus*, Cohn.

of the corrugations and the entire absence of mealiness affording distinctive characters. In color it is usually quite as distinct. Rarely, it remains for a time of a pale yellowish-white hue; but as a rule, even before the surface has begun to wrinkle, it passes into a clear lemon-yellow.

Within two or three days after its first appearance the zoogloea has usually passed its prime and changed to a yellowish-brown, which soon deepens to a very dark brown, with such an admixture of red as to render it almost a dull maroon. This color persists indefinitely, even on specimens which have been dried *in situ*.

The cells of this species vary from short ellipsoids to rods with their diameter and length in the ratio of 1:10 (Fig. 2); the measurements being $.35–.5 \times .8–3\ \mu$. The short articles are evidently unicellular. Those which are longer are sometimes constricted at equal intervals into two or four segments; sometimes, as with other species, apparently undivided. In old zoogloeae the cells are regularly shorter, forming the so-called gonidia, or their protoplasm collects at the poles preparatory to the formation of spores, which are similar in their arrangement and appearance to those of the last species.

In the connection already referred to,[1] Fresenius speaks of bright yellow zoogloeae on potato, which may have belonged to the species under consideration; but there are several things which may give rise to an appearance such as he describes. According to Wernich,[2] *Bacterium termo* sometimes forms a yellow slimy coating, often 2–3 mm. thick, on the same substratum. *Sarcina ventriculi*, a related plant frequent in certain diseases of the stomach, as well as in other fluids, may also grow on potato, where it forms chrome-yellow masses; while on vegetable and animal infusions it may appear as a yellowish surface film.[3]

While the reference of Fresenius is too vague to admit of comparison, our species may be distinguished from those forms of *B. termo* which I have seen by its wrinkled zoogloea and

[1] Fresenius: Beiträge zur Mykologie, p. 80.

[2] Wernich: Cohn's Beiträge, III, pp. 107, 118.

[3] Cohn: Beiträge zur Biologie der Pflanzen, I, Heft 2, p. 139; Schroeter: *op. cit.*, p. 119, note; Zopf: Die Spaltpilze, p. 91.

slightly different cells; and it differs greatly in the latter character from *Sarcina.*

Schroeter[1] found two pigment bacteria which more nearly approach our form. One of these, for which he proposed the name *Bacteridium luteum*,[2] and which was subsequently removed to the genus *Micrococcus* by Cohn,[3] was obtained on cooked potato, where it formed small yellow drops, and showed itself susceptible of culture in fluid media. The other species, which Schroeter names *Bacterium xanthinum*,[4] but considers identical with that previously called *Vibrio synxanthus* by Ehrenberg,[5] has been repeatedly observed on cooked milk in Europe,[6] but can also be grown on potato, etc., where it forms small lemon-yellow zoogloeae. Zopf regards these two species of Schroeter as probably forms of a single species.

Measurements are not given in the descriptions of *M. luteus*, but its cells are said to be ellipsoidal and somewhat larger than those of *M. prodigiosus*. Specimens distributed under this name by Winter[7] show round, somewhat ellipsoidal and oblong cells, measuring .8 × .8, 1.4 × 1.4 or .8–1 × 1.3–2.8 μ (Fig. 17). Presumably the latter belong to *Bacterium termo*, as the specimen was taken from rotting potato. *Bacterium synxanthum*, which is said to be morphologically identical with *B. termo*, so far as its cells are concerned, is .7–1 μ long. The diameter of the cells is not given, but comparisons which are made with other species, *e. g. B. lineola*,[8] would place it at about 1 μ, so that it must agree closely with *Micrococcus luteus* in this respect, as I have found it to do with what passes for *B. termo*. It will be seen, therefore, that the cells of both of Schroeter's species have a diameter nearly three times as great as those of that which I have cultivated.

[1] Schroeter: *l. c.*, p. 119–122. [2] *l. c.*, p. 126.
[3] Cohn: Beiträge, I, Heft 2, p. 153. [4] *l. c.*, p. 120.
[5] Ehrenberg: Ber. Verhandl. Berlin. Akad., 1840, p. 202.
[6] *Cf.* Zopf: Die Spaltpilze, p. 96. The species which produces a blue pigment in milk also forms yellowish or greenish-yellow zoogloeae on solid media, according to Hueppe (*cf.* Zeitschr. f. wiss. Mikroskopie, II, 113), but these differ in their microscopic characters.
[7] Von Thuemen: Mycotheca Universalis, No. 1400.—Helvetia, Zurich, in *Solani tuberosi* L., tuberis putridis. Jan. 1879.
[8] Winter: Rabenhorst's Kryptogamen Flora, I, p. 52.

While writing this paper I have received Poulsen's abstract of a paper by Rasmussen,[1] in which an unnamed *Bacterium* is said to form a yellow coating on culture media, on which it spreads rapidly. The cells of which this is composed are .6–.8 μ long.[2] *Micrococcus luteus* is said to have been frequent in the same series of cultures, so that it must have been distinguished from the *Bacterium*, which is distinctly said to be different from *B. xanthinum*, though it is designated simply as "No. 2." Without a fuller description it is impossible to say whether this is identical with our form or not; but it is so similar in the character of its cells that this may prove to be the case.

In this connection attention may be called to the somewhat different *Polybacteria sulfurea* of Van Tieghem,[3] which is said to consist of rounded or angular groups of sulphur-yellow cells, forming a surface film on an infusion of decaying beans.

Bacterium chlorinum (Cohn ?) Trelease (Fig. 16).

This species appeared as a number of greenish-yellow points on boiled potato, in a culture of the Baltimore material, started for *B. aurantiacum*, and was afterward encountered in some of the Cambridge specimens. Transfers gave rise to zoogloeae of 1–2 sq. cm., which, like the original drops, were at first glistening and slightly moist, but soon became waxy and dull on the baked potato on which they were grown. Specimens that were under observation for over a month preserved the same greenish-yellow color, which merely deepened a little with age. Their surface remained uniform, without any wrinkling. The cells of which the masses consist are shorter than in either of the preceding species of *Bacterium*, being broadly ellipsoidal or short cylindrical in form (Fig. 16). The measurements noted lie between .4 × .8 and .5 × 1.6 μ.

Schroeter appears to have been the first to notice green zoogloeae, during his work on pigment bacteria; but they were very poorly developed, so that nothing but a discoloration of the

[1] Rasmussen: Om Dyrkning af Mikroorganismer fra Spyt af sunde Mennesker. Dissertation, Copenhagen, 1883. Abstract in Bot. Centralblatt, XVII, Nos. 12, 13.

[2] Bot. Centralblatt, 1884, XVII, p. 416.

[3] Van Tieghem: Bull. Soc. Bot. de France, 1880, XXVII, p. 150.

potato they appeared on was seen.[1] Shortly afterward, Cohn obtained what he considers the same thing on cooked eggs, and in albumen which had undergone putrefaction, from which it was transferred to other culture media. Without giving an adequate description of it, Cohn named the species *Micrococcus chlorinus*,[2] placing it among his sphaerobacteria.

Subsequent writers have added little to our knowledge of these green zoogloeae. Zopf[3] states that the species of Cohn sometimes occurs in pus,—apparently on the authority of Girard or Gessard,[4] whose papers I have not seen; and Rasmussen[5] also speaks of a yellowish-green "*Bacterium* No. 1," with somewhat fusiform cells, .6–.8 μ long, as possibly identical with it.

Although the cells of our species are not much elongated, it is properly a *Bacterium;* but there seems to be some probability that it does not differ specifically from the *Micrococcus chlorinus* of Cohn, so that his specific name is retained.

Other green bacteria are *Bacterium aeruginosum*, Schroeter,[6] which has been found in bluish-green pus, and may have some connection with *Micrococcus pyocyaneus*, Gessard (*l. c.*); and two species found in impure water by Van Tieghem,[7] which were named by him *Bacterium viride* and *Bacillus virens*. It should be noted, however, that the latter species is apparently more properly one of the Cyanophyceae than a bacterian, as it is closely related to certain minute forms of this group which are questionably known under the names of *Leptothrix*, *Hypeothrix*, *Sporonema*, *Oscillaria*, etc.

Bacterium violaceum Bergonzini (Fig. 9).

On several occasions I was kindly supplied with material of a

[1] Schroeter: Cohn's Beiträge, I, Heft 2, p. 122. A green or yellowish-green discoloration is also caused in solid media, when these are used for the cultivation of the blue-pigment species of milk, according to Hueppe. *Cf.* Zeits. f. wiss. Mikrosk., II, 113.

[2] Cohn: Beiträge zur Biologie der Pflanzen, I, Heft 2, p. 155.

[3] Zopf: Die Spaltpilze, p. 90.

[4] Girard: Chir. Centralblatt, 1875, II, p. 50. Gessard: De la pyocyanine et de son microbe. Thesis, 1882.

[5] Rasmussen: Om Dyrkning af Mikroorganismer, etc., *fide* Bot. Centralblatt, XVII, p. 416.

[6] Schroeter: Cohn's Beiträge, I, Heft 2, p. 126; Cohn: *op. cit.*, p. 178.

[7] Van Tieghem: Bull. Soc. Bot. de France, 1880, XXVII, p. 174-9. A green form found in water is also mentioned by Hueppe, *l. c.*, p. 114.

brilliant violet zoogloea by Dr. Farlow, in whose laboratory it appeared spontaneously. Cultures were started with difficulty, from the fact that this species, like *Micrococcus prodigiosus*, passes through a short incubation period, during which other forms crowd in unless the most scrupulous care is exercised to exclude them.

Unlike most of the species mentioned in this paper, this is very sensitive to the light, and it is not uncommon to find it well developed on the lower side of a piece of potato, which gives no indication of its presence above. The zoogloea, which is smooth and waxy, like that of the last species, consists of very small oblong cells, measuring .3–.4 × .6–1.6 μ (Fig. 9).

What appears to be the same thing was found on white-of-egg by Bergonzini,[1] who describes its cells as measuring .6–1 × 2–3 μ, and gives it the name which I have applied to the American species, in spite of the smaller size of the latter. Zopf also describes a violet species under the name of *Bacterium ianthinum*,[2] which does not appear to be sufficiently distinct.

The only other violet species is one which Schroeter[3] found on potato, and which he named *Bacteridium violaceum*.[4] Its cells are said to be elliptical, frequently joined together in chains. Schroeter's cultures do not seem to have succeeded well, and Cohn, who transferred the species to the genus *Micrococcus*, had not cultivated it; nor do I find any record of further observations on it, although it is mentioned in all of the recent attempts at a systematic arrangement of the Schizophytes. While it cannot be said to be certain that the forms found by Schroeter and Bergonzini are distinct, the different solubility of their pigments, mentioned by the latter, and the apparent difference in their cells, is a sufficient warrant for holding them apart until they can be shown to be identical.

The only other species which form pigments at all similar to that of this species are the bluish-green species already referred to as occurring in pus, and a blue species found in milk;[5] but neither of these can be confounded with the violet forms.[6]

[1] Bergonzini: Annuario, Soc. dei Naturalisti in Modena, 1880, XIV, p. 149. Abst. in Bot. Centralblatt, 1880, p. 1528–30. [2] Zopf: Die Spaltpilze, p. 68.

[3] Schroeter: Cohn's Beiträge, I, Heft 2, p. 124. [4] *l. c.*, p. 126.

[5] For literature see Zopf: Die Spaltpilze, p. 58.

[6] Hueppe (*l. c.*, p. 114) mentions a species found in water, which produces a violet or blue-black pigment, and may be identical with our plant.

Bacterium incarnatum Trelease (Fig. 3).

Two brownish forms were found first in Baltimore, and again in Madison and Cambridge. One of these was of a deep brownish flesh color; the other was much paler.

On a moist boiled potato the development of the zoogloea begins soon after inoculation. At first, like several of the other species referred to, it spreads under these conditions as a watery slime nearly devoid of color. Its later growth is usually less rapid than that of *B. candidum*, *luteum* or *aurantiacum*; but within a few days, if conditions are favorable, it has assumed a smooth form much like that of the last-named species, and a similar waxy texture. Meanwhile its color deepens, until finally a shade is reached varying in the different cultures from dirty flesh-color through a brownish pink to a deep red-brown. The final color persists for a long time. As in *B. luteum*, the depth of color is mainly a matter of age, though the very pale and very dark forms in both appear to a certain extent to transmit their color peculiarities, irrespective of the condition of the potato on which they are propagated.

The cells composing these masses (Fig. 3) are much elongated and not obviously segmented; or shorter, when they have a broadly ellipsoidal form. Measurements range between .35 and .45 μ for their diameter, and .8 and 2 μ for their length. The cells of old zoogloeae contain spores .32–.4 μ in diameter, not distinguishable, so far as I could see, from those of the species already described.

No description fitting this species has been found. *Bacillus erythrosporus* Cohn,[1] which appeared in water containing albuminoids, and *Beggiatoa roseo-persicina* (Kütz.) (*Clathrocystis roseo-persicina* Auct.),[2] which is extremely common on salt marshes along the coast in summer, and causes considerable trouble by inducing a slimy decomposition of salted codfish,[3] although they may be red in one or all stages of their development, are very different. Cohn[4] gives a description, accompanied

[1] Cohn's Beiträge, I, Heft 3, p. 215–6 (Eidam); Miflet: *op. cit.*, III, p. 128; Winter: Rabenhorst's Krypt. Flora, I, p. 56.

[2] For literature see Zopf: Die Spaltpilze, p. 79–80.

[3] Farlow: Rept. U. S. Fish Comm., 1878, p. 969–973; 1879, p. 29.

[4] Cohn: Beiträge zur Biol. der Pflanzen, I, Heft 3, p. 181, pl. 6, f. 18.

by figures, of *Micrococcus fulvus*, which occurs in rusty red drops on horse dung; but his account and specimens distributed by Kirchner[1] do not agree with the form on potato. *Micrococcus prodigiosus*, mentioned above in this paper, is also very different, though of a red color. Several plants, at present classed with the Saccharomycetes, will be considered later as forming red masses; but all are very dissimilar to the species under consideration.

Schroeter[2] noticed a brown pigment in a decoction of corn and wheat seed as well as in water that potatoes had been cooked in. The former contained quiescent cells; the latter swarmed with a Bacterium and long Vibrios. Without characterizing it he suggests[3] that a *Bacteridium brunneum* is probably to be recognized in addition to the other pigment species.

Cohn[4] and Frank describe and figure a plant under the name of *Bacillus ruber* which was obtained on cooked rice, on which it formed a vermilion or brick-red coating. Measurements are not given, but from the figure it appears that the cells of this species are slender, their length being 5 to 35 times their diameter. Specimens distributed by Dr. Frank,[5] the discoverer of the species, vary from broadly ellipsoidal to linear, thus agreeing very nearly in form with our plant; but they are at least twice as large, measuring $1–1.6 \times 3.2–7.2\,\mu$, in the specimen examined (Fig. 15). Winter,[6] who apparently studied the same exsiccata, gives the diameter of the cells as nearly 1 μ, while their length is 3–4 or even 6 or 8 μ.

Until this difference in size has been shown not to be characteristic, it appears sufficient, in connection with the difference in color, to warrant the separation of our form as a perfectly distinct species. It will be understood that neither of these species has anything in common with *Cladothrix*, *Crenothrix*, etc., which are colored brown by a deposition of hydrate of iron in their walls; since they owe their color to a true organic pigment of their own formation.

[1] Rabenhorst's Alg. Eur., No. 2501.
[2] Schroeter: Cohn's Beiträge, I, Heft 2, p. 125; Cohn: *l. c.*, p. 157.
[3] *l. c.*, p. 126.
[4] Cohn: Beiträge zur Biol. der Pfl., I, Heft 3, p. 181, pl. 6, f. 17.
[5] Rabenhorst's Alg. Eur., No. 2441.
[6] Winter: Rabenhorst's Krypt. Flora, I, p. 56.

Bacterium hyalinum Kütz (Fig. 11).

A minute colorless plant was found at Madison, by Mr. L. H. Pammel, among various Chroococcaceae, Palmellaceae and Desmids, in ditch water. Its cells vary from nearly round to broadly oblong, and are by no means uniform in size, measuring .8 × 1.2 μ—1.5 × 2 μ. They divide in two planes at right angles to each other, so as to form ultimately small fronds consisting of a single layer of cells, as in *Merismopedia*. The division of the different cells of the same set is not synchronous, however, as in that genus; hence the frond is not usually composed of as regular tetrads (Fig. 11).

In its general characters this plant approaches several forms which are usually classed with the Cyanophyceae, under the genera *Merismopedia* and *Sarcina*. So far as can be seen it is identical with that known as *Merismopedia hyalina*, Kütz.[1]

Zopf has recently shown[2] that in this species the cells divide in but one plane, at certain seasons, thus forming moniliform threads containing, now and then, elongated cells. Irregular zoogloea masses are also figured by him. In view of these facts the species has been named *Bacterium merismopediodes* by Zopf.

Although only merismopedioid colonies and isolated cells have been found at Madison, there is little doubt of the identity of the American and German forms; but in deference to the views of Winter and others on synonymy, I have retained for the species the specific name given it by Kützing, while placing it in the genus to which Zopf transferred it.

Cladothrix dichotoma Cohn (Fig. 5, 12).

While examining a fresh-water *Cladophora*, collected at Cambridge, in the fall of 1881, I noticed that many of its cells were covered by small colorless bodies, 5–10 μ long and 2–5 μ in diameter. Their form suggested certain species of Palmellaceae of the genus *Characium*, which are common in similar situations; but a sufficiently high power showed them to be in reality small

[1] Kützing: Tab. Phycolog., V, pl. 38, f. 1.

[2] Zopf: Sitzber. bot. Vereins Prov. Brandenburg, June, 1882; Die Spaltpilze, p. 56, f. 19.

zoogloea masses, composed of colorless cells, .3–.5 μ in diameter, and usually 1.5–2 μ long, which lie in regular succession—the result of transverse division (Fig. 12). Some of these bodies were more or less broken, the liberated cells swimming about singly or in short chains, but I was unable to see the latter in the act of escaping. The zoogloeae were sometimes accompanied by leptothrix filaments, of about the same diameter as their cells and of different lengths, which appeared to be forms of the same species.

The same season some mud from a pond at Woods Holl, Mass., was kept under observation for several months. Among the Desmids and other minute algae which grew in the water covering it, many dendritic brown bodies, .1–.2 mm. long, were found (Fig. 5). These bodies, which were also zoogloeae, consisting of ovoid cells not far from .3 μ in diameter and 1 μ long, agree perfectly with what is known as *Zoogloea ramigera* (Itzigs.),[1] which is now recognized as a form of *Cladothrix dichotoma* Cohn.[2] The characioid zoogloeae are similar to one figured in 1877 by Cienkowski, and shown to belong to the *Cladothrix* just mentioned.[3]

The brown color of the branched zoogloea is due to a deposition of iron hydrate, which also colors a related species—*Crenothrix kuhniana* (Rabh.)—that has been quite troublesome by forming flocculent brown masses in the water-jars and wash-bottles of my laboratory for the last year or two.

Typical *Cladothrix* threads, showing the false branching characteristic of the genus, were not noticed in connection with either of the described zoogloeae; but they have been repeatedly observed both at Cambridge and Madison, the species being, as in Europe, the commonest of fresh-water bacteria.

Leptothrix buccalis Robin (Fig. 7).

For several years I have been a forced observer of certain small zoogloeae that form in the vicinity of the human pharynx, especially during a "cold in the head"; apparently living on

[1] *Cf.* Koch: Cohn's Beiträge, II, Heft 3, p. 414, pl. 14, f. 1–2.

[2] Zopf: Die Spaltpilze, p. 85.

[3] Cienkowski: Mém. Acad. St. Pétérsb., XXV, pl. 1, f. 24; Zopf: Zur Morphologie der Spaltpflanzen, I, Die Spaltpilze, pp. 28, 83.

the discharged mucus. These bodies often cause an irritation similar to that occasioned by a crumb lodged near the posterior nares, and their protected position generally renders their removal difficult, a vigorous and prolonged rasping of the throat alone sufficing. Sometimes only one can be removed: at other times, especially after a protracted cold, several are dislodged.

The fresh zoogloea (Fig. 7*a*), which is well marked in the uniformity of its characters, is 1–3 mm. in diameter, rounded, but divided into several large lobes by shallow sinuses. Its surface is uniformly and finely granular under a lens. In color it may be described as dirty-white, and its texture is farinaceous, much like that of germinated malt at the time it is killed. It emits an extremely disagreeable odor, like that of teeth affected with caries. Left exposed to the air it quickly dries into a yellow horny mass, which soon becomes quite odorless.

As far as I can determine, these bodies do not cause the catarrhal affection they accompany, but live merely as saprophytes on the abnormally abundant secretion of the inflamed mucous membrane, though their presence appears to prolong the disorder. The sinuses referred to apparently indicate the commencement of division, by which the number of the bodies is increased.

Several morphologically distinct cells have been noticed in these zoogloeae. That which is most abundant and apparently characteristic consists of cocci or rods, .25 μ in diameter (Fig. 7*b*, *c*). The rods, which occur singly or in chains, vary in length from 1–3 μ. Both forms agree with states of the common *Leptothrix* of the mouth, which has been shown to be the cause of decay in the teeth,[1] and the pharyngeal zoogloeae are unquestionably a well-marked form of that species.

2. Saccharomycetes.

Saccharomyces glutinis (Fres.) (Fig. 6).

During my cultures of bacteria, a semi-fluid claret-colored drop was detected on some corn-starch paste which had been inoculated with *Micrococcus prodigiosus*. Some of this drop was

[1] For a very good account of this species, with references to important papers, see Zopf: Die Spaltpilze, p. 80–83.

promptly transferred to baked potato, where it developed rapidly, forming an irregular granulated heap a little under 1 cm. in diameter and 1–2 mm. thick, of a decided red color, somewhat different from that of the original drop. The thicker parts soon dried at the surface, becoming somewhat crumbly and paler, when their appearance was such as to recall dried bits of the carmine plaster-of-Paris injecting-mass sometimes used by anatomists. The plant obtained in this way maintained its characters unchanged through many culture generations; but it spread more rapidly and retained more moisture when grown on boiled potato than when the drier baked potato was used, and in cases of luxuriant development was of a pure vermilion color. In one culture, with a night temperature considerably below 60° F., and a day temperature of 68°–70° F., it had spread over the entire cut surface of half a large potato, from a few small centres of inoculation, in less than five days. In some places it was uniformly smooth, but in the main presented a slightly mammillated appearance that I had before noted as rather characteristic.

This red substance consists of isolated cells 2–5 μ in diameter (Fig. 6), nearly globular, or slightly ellipsoidal where growth is active. Each cell has a thin colorless wall, enclosing a mass of homogeneous protoplasm that either entirely fills it or forms a parietal layer about one or two vacuoles. In old or diseased cells the protoplasm is usually granular. When vacuoles are present they are generally excentric. A dot of protoplasm, more refractive than that about it, can usually be seen in the thicker part of the parietal layer, or somewhere near the wall when there are no vacuoles. Sometimes there are two of these nucleoid bodies, which are .3–.8 μ in diameter.[1]

At some point, which apparently has no relation to the position of the nucleoid dot or the vacuoles, a hemispherical protrusion appears on the surface of the cell. At first measuring scarcely .5 μ, this grows until it reaches a diameter approximating that of

[1] These are well shown in Cohn's figure of this species—Beiträge, I, Heft 2, pl. 3, f. 6. On the doubtful occurrence of true nuclei in yeasts see Schmitz: Sitzber. niederrh. Ges. für Nat.- und Heilk., August 4, 1879, and Just's Bot. Jahresber., 1879, Abth. I, p. 8; and Strasburger: Zellbildung und Zelltheilung, 1880, p. 672.

the original cell, meantime rounding off until it finally separates as a new cell. Usually the separation occurs when it has reached a diameter of about 2 μ, so that it is not common to find two large cells adherent. More than two have not been seen united. A nuclear granule may be detected in the young cell soon after its appearance as a bud, in some cases; but in others no trace of it is to be found.

This plant, which is characterized as a yeast by its budding, agrees perfectly with the form known in Europe as *Saccharomyces glutinis*.[1]

In a thesis on the organisms of beer and beer-wort, Hansen[2] mentions three red ferment fungi.[3] One of these is identical with the Fresenian species; a second, which produces ascospores, is held to be distinct; while the third, which does not form spores, is said to develop a sort of mycelium when insufficiently nourished, this, as well as the mother-cells, budding when transferred to a good culture-fluid. From this it will be seen that several species have apparently been confounded under the specific name given by Fresenius; but, so far as can at present be determined, the American species is identical with that to which Cohn applied this name in removing it from the old genus. During the past winter, a form of the same species was found on rotting turnips, thriving in a cellar at a temperature little above the freezing point. Its cells resemble closely those figured by Fresenius.

The remarkable reddish *S. coprogenus* Sacc. & Speg., which forms a waxy layer on fermenting human excrement,[4] should be mentioned in this connection, though entirely different from the plant under consideration.

Saccharomyces glutinis (Fres.), var. *candidus* Trelease (Fig. 6 *a*).

Some time before the typical *S. glutinis* was observed, several white spots appeared on a slice of boiled potato, near a culture

[1] Fresenius: Beiträge zur Mykol., pp. 77–8; Schroeter: Cohn's Beiträge, I, Heft 2, p. 110, note; Cohn: *op. cit.*, pp. 187–8, 209, pl. 3, f. 6, etc. It should be said that Fresenius' figures (pl. 8, f. 43–6) show more elongated cells than those of Cohn.

[2] Hansen: Organismer i öl og ölurt, Copenhagen, 1879, p. 62–75, pl. 2, f. 1–44.

[3] *Cf.*, also, Rasmussen's paper referred to above, and the abstract in Bot. Centralbl., XVII, p. 391–2.

[4] Mycotheca Veneta, No. 1584; Fungi Italici, f. 911; Michelia, II, p. 287.

of *Bacterium aurantiacum.* They were at first less than .5 mm. in diameter, nearly hemispherical, and moist. In a few days a number of these spots, now measuring 1 mm., had become confluent, forming a mass about 7 mm. in diameter, but not above 1 mm. thick; while others, situated at a little distance, remained distinct and maintained their hemispherical form. Meantime their surface had become dry, and presented a chalky appearance. It was also regularly roughened by low rounded granulations. Particles of the chalky mass crumbled to powder under the slightest pressure.

The characters of this form were perpetuated unchanged through a number of transfers; but it was observed that on soggy boiled potato development was more rapid, so that a half potato was nearly covered in a few days, when kept at a suitable temperature, nor did the mass become so dry and pulverulent. From first to last it presented a close resemblance to *Micrococcus candidus*, as this was subsequently observed.

The microscopic characters of these white specimens are identical with those of *Saccharomyces glutinis.* They consist of the same round or slightly elongated cells, which possess nucleoid dots, which may or may not have their protoplasm interrupted by vacuoles, and which bud precisely like those of the red yeast, with which they also agree in size (Fig. 6 *a*).

Notwithstanding the apparent morphological identity of this plant with the species of Fresenius, it constantly remains colorless, while the latter develops its pigment, when both are grown on halves of the same potato and under precisely similar conditions. This feature, the constancy of which renders its identification easy, characterizes it as a distinct species, or, as I prefer to consider it, a well-marked variety of *S. glutinis.* It is presumably a *Torula* in the sense of Pasteur and Hansen.[1]

At one time a dirty-white waxy spot, about 5 mm. in diameter and one or two millimeters thick, was noticed on a slice of steamed potato. In some respects it resembled the white yeast just described; but, aside from its color, it was readily distinguishable by the peculiar appearance of its surface, which,

[1] *Cf.* Hansen: Meddelelser fra Carlsberg Lab., II, 2, 1888, p. 48 *et seq.* of resumé.

covered by salient points, resembled the hymenium of certain species of *Hydnum*.

The microscope showed that it was composed of two apparently distinct parts: 1, a yeast somewhat larger than *S. glutinis*, and differing from it in the more irregular form and greater coherence of its cells (Fig. 4); and 2, a segmented mycelioid growth, which, however, was readily separable into its component cells (Fig. 14). This was evidently a form of *Oidium lactis* Fres., a species very common in sour milk and in the film on the surface of souring fluids.[1] The growth of its threads, carrying up the surrounding yeast cells, was undoubtedly the cause of the toothed surface of the mass; but the material was lost before it had been sufficiently studied. Similar pointed outgrowths are figured by Hansen.[2]

DESCRIPTION OF THE PLATES.

FIG. 1. *Bacterium candidum* Trelease, from specimens grown from Baltimore material. *a*, beginning spore formation. *b*, cell as seen from the end (× 2500).

FIG. 2. *Bacterium luteum* Trelease, long and short rods, cocci and spores, from cultivated specimens (× 2500).

FIG. 3. *Bacterium incarnatum* Trelease, from Madison cultures—vegetative cells and spores (× 2500).

FIG. 4. *Saccharomyces* sp., occurring with *Oidium*, in hydnoid mass on potato (× 2500).

FIG. 5. *Cladothrix dichotoma* Cohn. "Zoogloea ramigera," cultivated from Woods Holl material. *a*, (× 425); *b*, isolated cells (× 2500).

FIG. 6. *Saccharomyces glutinis* (Fres.). *a*, from var. *candidus* (× 2500).

FIG. 7. *Leptothrix buccalis* Robin, from human pharynx. *a*, zoogloea (× 4). *b*, rods, cocci and spores. *c*, larger rods and spores from the same zoogloea (× 2500).

FIG. 8. *Bacterium aurantiacum* Trelease. *a*, from living specimens. *b*, from the same slide, stained with methyl violet and mounted in benzol-balsam. *c*, *d*, sporiferous (× 2500).

FIG. 9. *Bacterium violaceum* Bergonz., from Cambridge material (× 2500).

[1] *Cf.* Cienkowski: Die Pilze der Kahmhaut.—Mélanges Biologiques, VIII, 1872, p. 566–592, pl. 1–2.

[2] Hansen: Organismer, pl. 1, f. 8.

FIG. 10. *Micrococcus aurantiacus* (Schr.), from one of Fischer's slides (× 2500).

FIG. 11. *Bacterium hyalinum* (Kütz.), from specimens gathered at Madison (× 2500).

FIG. 12. *Cladothrix dichotoma* Cohn. Characioid zoogloeae gathered at Cambridge (× 2500).

FIG. 13. *Micrococcus prodigiosus* (Ehr.), from slide by Fischer, agreeing with Cambridge specimens (× 2500).

FIG. 14. *Oidium lactis* Fres., from hydnoid zoogloea (× 570).

FIG. 15. *Bacillus ruber* Cohn and Frank, from specimens in Rabenhorst's Algae Europaeae (× 2500).

FIG. 16. *Bacterium chlorinum* (Cohn ?) Trelease, from specimens cultivated at Madison (× 2500).

FIG. 17. *Micrococcus luteus* (Schr.), from specimens in Mycotheca Universalis (× 2500). Apparently with *Bacterium termo.*

DEVELOPMENT OF THE GILL IN FASCIOLARIA. By HENRY LESLIE OSBORN, PH. D., Instructor in Zoology in Purdue University, Lafayette, Indiana. With Plate XIII.

The study here presented is based upon a form determined by Prof. G. W. Tryon, Jr., as *Fasciolaria tulipa*, Linn. var. *distans* Lam.[1] The material for study was obtained during a season at the Chesapeake Zoological Laboratory in 1884. All the surface views were obtained from living specimens, and these, preserved in chromic acid .2 per cent. 24 hours, and then passed through serial alcohols to 70 per cent., remain permanently in fine condition for surface observation. For sections embryos preserved in corrosive sublimate, saturated solution, half an hour, then washed in water one hour, or picro-nitric acid, Meyer's formula gave the best satisfaction.

The creatures are very abundant at Beaufort, North Carolina, living on the mud flats, between tides in shallow sounds, and their egg capsules can be abundantly found. In a future paper on the development of *Fasciolaria* I shall describe these capsules and their situation, but for the present purpose it is enough to say that the eggs develop within these capsules, and that the development of an individual egg cannot be followed, since the eggs cease to develop as soon as removed from the capsules. In view of this fact, we cannot be positively certain that the successive changes are as here described, but there is no rational doubt that it is so, as determined by the examination of a large number of specimens in all stages of development. Therefore while I describe the changes as if succeeding one another in one egg, the facts observed are drawn from a large number of eggs.

The form of body when the gills first appear, is represented in Fig. 1. This shows the body as seen on the left side, and with

[1] In an abstract of this work presented in Johns Hopkins University Circular, No. 85, this animal was improperly called Neptunea; this error, I seize the present opportunity to correct.

the foot and velum of the left side uppermost. This mass underlying the velum is composed of huge non-nucleated deeply staining bodies, which are later completely absorbed before the disappearance of the velum, and which are doubtless supplementary food masses. They are, so far as I am aware, undescribed, and I shall call them the "*sub-velar masses.*" Partly encircling the body runs a ridge like a girdle, and between the girdle and the head region lies a row of folds of the ectoderm in that region. This region is the region of the ectoderm that lines the future mantle cavity. It has been derived from cells which began first as a small circular ring on the side of the body opposite the foot, and enlarged till it spread over the whole dorsal surface. This surface was previously enclosed in ectoderm; the thickening was a differentiation in the ectoderm cells which progressed outwardly, growing larger and larger as it traveled over the dorsal surface. It is illustrated in Fig. 2.

Long before there is any trace of the gills a heart rythmically contractile is observed. It appears upon the right side of the body just behind the sub-velar masses and in front of the region to be occupied by the future gill.

In Fig. 3 a view looking down upon the mantle surface is shown. The advancing edge of the mantle, the mass which girdles the embryo in Fig. 1, is shown at *mn.*; in front of this lies the corrugated skin, which is the very simple gill, and in front of this, not shown in the figure, lies the pulsatile heart. Here we have the simplest form of gill that can be found among the prosobranchiate gastropods. At this time the internal differentiation has gone so far that the stomodaeum and proctodaeum are established, the gut walled in, the nervous ganglia of the head, foot and olfactory organ begin to show.

In Fig. 4 a sagittal section of the embryo through the gill is represented. The section is to the right side of the middle line. Here can be seen the velum and sub-velum in position and free here from the body, the gill upon the dorsal surface (which is at present downward) in front of it, the heart a space in the mesoderm, opposite this the edge of the mantle which is to form the future free edge of the same. The gut is at this time packed full of food yolk, and the endoderm cells are not as yet developed as digestive in all its parts. In Fig. 5 cross sections of two of the

gill folds are shown, and the cells are drawn as nearly true as to shape as possible but exaggerated in size for convenience in drawing. It may be seen that the only differentiation they exhibit in structure is the ciliated cells; the other cells are almost precisely like those of the general surface. This is the simplest form of gill thus far described as existing among the gastropods. It is what we should anticipate as a most elementary form of gill, both from comparison with adult ctenobranch gills and from the gills of the lamellibranch, as we have learned to understand them, but it is simpler than either of these, for it is here found upon the outer surface of the body and not even enclosed in a mantle chamber.

The next step in the development is illustrated in Fig. 6 and Fig. 7, surface views, the latter later than the former, and in Fig. 8 a sagittal section through an embryo in about the stage shown in Fig. 7. The form of body is already characteristic, the shell has been deposited and has taken upon itself, beginning from the region behind the foot, the characteristic shape, it is drawn out into a beginning of the columellar portion upon the left side, the portion finally occupied by the spire is now rounded and the edge of the shell on the dorsal side of the creature is spreading forward so as to cover in the body; but at present it has not gone so far as this, and the dorsal surface of the body is not yet furnished with that peculiar mantle or branchial chamber. The gill area of the preceding stage has, however, undergone a great change; it is beginning to roll inward by an involution commencing near the head, the gill at first lying upon a convex surface is pulled inward, as it were, from inside, and the area it occupies is nearly flat, the gill being curved to allow its lying crowded into so small an area.

Fig. 8 shows a section cut from the same embryo as is sketched in Fig. 7. The edge of the mantle, the shell, the foot, the pedal ganglion, the gut with huge vacuolated digestive cells, their outlines not preserved, and finally the crumpled gill, in front of the edge of the mantle, and behind the region of the head, are the features to be noticed. Here is strictly an intermediate condition between the embryonic condition and the definitive condition of the gill. The advancing differentiations would make it undoubted that it follows the first stage, even were not the next

and final stage so obviously subsequent. There can be no doubt, from the study of the progressive changes in other parts of the body, that the succession of the change as here described is the true order of development.

In Fig. 9 a section similar to Figs. 4 and 8 is illustrated, taken from a specimen which had assumed the adult shape. Viewed externally, the vela are still seen too large to be wholly drawn into the shell; traces of the sub-velar masses are still present. The foot is now used in locomotion, and the young snail creeps about in the watch glass, the mantle is wholly folded in, and no trace of the gill can be seen from the exterior. In the section the shell is seen surrounding all with the operculum beneath the foot. The mantle cavity is now fully formed and occupies the usual position on its inner surface; the gill is found, and it now shows some interesting differentiation in its epithelium connecting it with the adult form. Behind the gill is the heart; it has kept pace with the changes in the gill and, from a position in front of the gill on the outside, it now has a position behind the gill, the normal adult position of the prosobranch heart. It is thus seen that the gill in Fasciolaria is directly derived from a series of simple folds of the ectoderm of the outer surface of the body.

The structure of the gill in the adult is not any different from that of *Fulgur* figured and described in a former paper.[1] It consists of a row of independent plates of triangular outline, hanging down into the mantle cavity from its roof. These plates abut at their left end upon a ridge, which, unlike *Fulgur* carries no definite bloodvessel, and they run transversely over toward the right. Running alongside the gill is the olfactory organ. It consists of a nerve trunk running along a central stem and plates arranged on either side the stem, somewhat like the gill plates. Figure 10 represents a surface view of a gill plate, and the two flaps of the olfactory organ and the nerve cut across the end. The epithelium of the gill plate shows in the sections three areas exhibited in Fig. 11. The outer portion consists of closely fitted columnar epithelium cells on the end of the plate, and in a band near the end, while just between these, barely covering

[1] Studies from the Biological Laboratory, Vol. III, Pl. IV, Fig. 10; Pl. V, Figs. 11, 12 and 13.

the basement membrane, there greatly thickened to form the chitinous supporting rod, the cells are much lower and not ciliated. Following the outer portion there comes a broad band composed of large, irregular, loosely placed cells, nucleated and without cilia, and these are followed by an inner area of very closely packed but non-ciliated cells. In sections of the gill at the age of the specimen figured as No. 9, the epithelium of the gill shows two sorts of cells, the outer row and the middle row, being about similar in shape at that time to the adult. These are represented in Fig. 12 *a* and 12 *b*.

I wish to reserve the description of the remaining embryonic history of *Fasciolaria* for a future paper, but will point out what I consider the importance of these facts of development to molluscan morphology. The development of *Fasciolaria* is very peculiar if not unique, so far as at present known among prosobranchs. I have studied *Fulgur*, and have all the stages from the youngest eggs up to the adult, but nothing at all like this mode of formation of the gill occurs in this closely allied form. Both pass their early stages in protective capsules, and yet the life histories are very different. In *Fulgur* the development is direct, and the gill where it arises is formed upon the wall of a mantle cavity. In *Crepidula* also the gill arises after the mantle cavity, and we do not know that in any other prosobranch the mantle cavity arises as it does in *Fasciolaria*.[1] The development in *Fulgur*, while it shows resemblance to that of *Fasciolaria*, and can be derived from it, is plainly a briefer course derived from a mode like that of *Fasciolaria*, but with certain steps in the process dropped out. There seems but little doubt that in *Fasciolaria* there is found a form of development which is more primitive for ctenobranchs than any yet described. There is nothing in the circumstances of development to lead us to the belief that the development here is secondary, but everything so far as known points to the view, that the ctenobranchs are from ancestors like the young *Fasciolaria*. We must believe that the

[1] Rable describes in Planorbis (Entw. Tellerschnecken, Morphol. Jahrbuch, Vol. V.) the mode of formation of the mantle cavity which is much like that of Fasciolaria. The space which is the difinitive mantle cavity, is at first uncovered at the exterior, and only later the growing edge of the mantle is drawn from behind forward so as to be opened over it.

mantle cavity was at first a shallow groove for them, and we can easily believe that the gill was at one time upon the outer side of the body. If we do not take this view for *Fasciolaria*, we must suppose that we have here a case of an animal which has lost and reacquired a structure, and while it shows no trace of the loss of the structure, recapitulates the history of its reacquirement. If it be urged that *Fasciolaria* loses the mantle cavity because not needed in its protected embryonic life, and acquires it only when it is to emerge from its protective capsule, then I should inquire why Fulgur and the others, so far as known, do not go through a similar course, for they spend their embryo existence under quite similar conditions.

Considering the question solely with the ctenobranch in view, the similarity of the gill in this group, and the fact of development so far as at present known, may be recapitulated thus: The ctenobranchs are characterized by a single gill which lies on one side of the body, it is a series of folds in the wall of the skin forming the mantle cavity, it lies in front of the heart, stretches forward, but if the fold that forms the mantle cavity were straightened out one should have for the ctenobranch gill a series of folds in the outer skin of the body lying behind the heart stretching backward. In the embryo of Fasciolaria precisely this condition is a normal stage in the ontogenetic development. The inference would be that this ontogeny repeated the phylogeny. That for the ctenobranchs the gill arose as a series of folds on the outer surface, a single series of folds median in position or nearly so, and behind the heart. That in later growth the gill was carried on further toward the left, and with it the heart which originated on the right side of the body, in consequence of the spiral twist that involves the dorsal portion of the body, and that it was also for purposes of protection perhaps folded into a cavity by a growth of the skin on which it arose. We should know that in the ctenobranch the gill is not a median structure, but one that properly belongs to the right side of the body, and has suffered translocation with the heart and visceral mass, from the innervation of the gill and attendant osphradium from the right side of the body.

This view would harmonize with the facts as known in the lamellibranchs. There the gills are paired, but, as shown by

Mitzukuri, are to be considered a simple ridge or elevation of the skin with folds along two sides of it.[1] The presence in a chamber is a secondary consideration in comparison with the main fact that the gill begins as a series of folds in the skin, and not as a rachis furnished with flaps on either side, which flaps secondarily fuse with walls of the mantle cavity.

Let us now proceed to examine the fact as to the molluscan gill outside the ctenobranchs and lamellibranchs. We find in the other forms, when present, a peculiar organ, which has been termed by Lankester a *ctenidium*. The gill of *Fissurella*, *Trochus*, *Chiton*, the Cephalophods is essentially a ridge penetrated by one or two bloodvessels and furnished on either side with a flap of skin surrounding a broad expanded network in communication with the bloodvessel in the rachis,[2] these flaps, and the rachis as well, at least on the portion furthest from the heart, being borne free from the mantle wall. In his Schematic Mollusk, figured by Lankester in the same article,[3] the gill is figured as thus described. Lankester does not indicate a way of comparing the ctenobranch gill with the ctenidium, or of showing its relation to that. But Spengel indicates that the present simplicity of the ctenobranch gill is secondary, and that a paired ctenidium preceded it in earlier forms. In his article on the olfactory organ of Mollusks,[4] Spengel remarks in a note that the gills are in the ctenobranchs to be derived from a true ctenidium, the blade on one side having been fused with the mantle wall and lost, while the blade of the other side remains as the present gill. I believe that the matter is made much simpler by the hypothesis that in the ctenobranchs the lost blade of Spengel never existed, that the ctenidium condition of the gill has never been reached at all in the most of the ctenobranchs, that we find a progress in that direction in some as *Sigaretus*, when the gill shows two sides which may be compared with the two blades, and where the tip is slightly free from the mantle, and that we have a true ctenidium in a few

[1] Mitzukuri. Studies Biol. Lab. J. H. U., Vol. II, p. 269.

[2] See E. Ray Lankester, Art Mollusca, Encyc. Britt. Ed. 9th, Vol. XVI, p. 638, *et seq.*

[3] E. Ray Lankester, *op. cit.*, p. 635.

[4] Spengel. Die Geruchsorgane der Mollusken, Zeitschr. f. W. Zool., Vol. XXXV, p. 355, *et seq.*

anomalous cases, as *Valvata*, not at present described fully or carefully enough to give us much available information.

Considering the gill then as the basis for classification, we could divide up the Mollusks into a series which would place those forms with the most completely developed ctenidia highest, or as the more specialized members. I believe that this would be true of the gill itself. I believe that the facts so far as we possess them in regard to the structure of the molluscan gill and its evolution would bring us to consider the ctenidium a specialized organ derived from a simple structure, such as is present in the young *Fasciolaria*. But I am far from thinking that it would be sound to put the ctenobranchs for this reason at the bottom of the prosobranch series, and the zeugobranchs above them as derived from them.

What bearing this history of the organ might have on the classification I should not care to discuss, until there are more facts discovered to prove this the true history of the molluscan gill. At present we know nothing at all of the development of the gill in those forms of gastropods which have a ctenidium, and more information there might lead us to regard with greater favor the conjecture of Spengel.

LIST OF FIGURES.

Plate XIII.

Figure 1. View of left side of *Fasciolaria*, seen shortly after the first appearance of the gill-folds.

Figure 2. View of dorsal surface of *Fasciolaria* when the shell gland has first appeared.

Figure 3. View of *Fasciolaria* in same stage as Fig. 1, seen looking down upon the dorsal surface. In front of the velum is a large transparent protuberant head, the remainder of the body is perfectly opaque, owing to the presence of an enormous amount of food yolk. The advancing edge of the mantle is seen at *mn*.

Figure 4. Sagittal section of embryo, in which the gill is still completely exposed. The gut is seen still filled with food yolk, with the passage leading to the anus lined with columnar ciliated cells and with large vacuolated digestive cells over a portion of the rest of its wall. The heart (*Ht*), the gill behind it (*Br*), the edge of the mantle (*mn*).

FIGURE 5. Two plates of Figure 4 more highly magnified to show epithelium.

FIGURE 6. View of ventral surface of embryo, in which the gill has begun to roll in.

FIGURE 7. View of somewhat later embryo looking down upon the dorsal surface.

FIGURE 8. Sagittal section of embryo figured in 7.

FIGURE 9. Sagittal section through gill and heart of embryo still within capsule, but having acquired external adult shape.

FIGURE 10. Surface view of single plate of full grown *Fasciolaria.*

FIGURE 11. Transverse section of four gill plates of *Fasciolaria* adult.

FIGURE 12 *a–b*. Epithelium cells from outer and inner portions of gill plate in embryo, Figure 9.

FIGURES 4, 5, 8, 9, 10 and 11 were drawn with the camera lucida, also the measuring scale which accompanies each figure. From these the actual size and degree of enlargement may be directly obtained.

ABBREVIATIONS IN ALL THE FIGURES.

Br. Gill.

Br. cav. Cavity in gill communicating with lacunar space through the mesoderm of the body.

F. Foot.

Fr. Transparent frontal lobe.

G. Endoderm portion of gut wall.

M. Mesoderm.

Mn. Mantle.

M. o. s. Mantle outer surface.

M. i. s. Mantle inner surface.

Nv. Olfactory nerve.

Ol. Olfactory organ.

Op. Operculum.

Pr. Proctodaeum.

P. Nv. Foot nerve.

Sh. Shell.

S. r. Supporting rod of gill plate.

S. v. Subvelar mass.

St. Stomodaeum.

V. Velum.

Y. Food yolk.

A STUDY OF THE STRUCTURE OF LINGULA (GLOTTIDIA) PYRAMIDATA STIM. (DALL).

By H. G. BEYER, M. D., M. R. C. S. (London), Passed Asst. Surgeon U. S. N., Honorary Curator Section Materia Medica, U. S. National Museum. With Plates XIV, XV, XVI, XVII.

INTRODUCTION.

The investigation into the minute structure of Lingula, described in the following pages, was begun in the autumn of 1883, in the Biological Laboratory of the Johns Hopkins University, at the suggestion of Professor W. K. Brooks, who very kindly placed at my disposal a large collection of this genus, gathered at Beaufort, S. C., and partly also at the mouth of the Chesapeake Bay.

Unfortunately, these specimens had all been hardened in chromic acid, and, after making several series of sections, it was found, while some of them were preserved very well indeed, others proved to be quite unfit for histological work, chromic acid being, apparently, not the best hardening agent for Lingulas.

Comparatively little was, therefore, done until March, 1884, when I was able to procure a fresh lot of specimens, some of which were put through the chromic-acid process, others through picric acid, again others through osmic and acetic acid, and so on. The results turned out to be very much in favor of the picric-acid process, and those specimens which were prepared in this way, and afterwards stained in picro- or borax-carmine, left nothing to be desired, so far as histological details are concerned. Hoping to supplement these histological studies by some embryological ones at the seaside, more especially with reference to the earlier stages, the later ones having already been worked out with much care and detail by W. K. Brooks, I have delayed publishing this work until now.

In order to determine the exact systematic position of this particular species of Lingula, I took some of the specimens to Prof.

Wm. H. Dall, of the Smithsonian Inst., and the following account is a description of the genus in his own words:

"*Glottidia pyramidata* (Stm.) *Dall.*

" This brachiopod was originally described by Dr. Wm. Stimpson under the name of *Lingula pyramidata* (*Am. Journ. Sci. & Arts*, XXXIX, p. 444, 1860), and was obtained by him in North Carolina.[1] In 1870, while engaged in the study of the Lingulas in the National Collection, I discovered that some species were distinctly separated from the typical form of the genus, in being provided with raised fulcra for the attachment of certain muscles, forming a median septum in one and two divaricating septa in the other valve. To this group I applied the name of *Glottidia*, which has been adopted. The type is a species very similar to southern specimens of *G. pyramidata*—namely, the *G. albida* of Hinds. There are three other closely related forms on the west coast of America, *G. semen* Brod., *G. audebarti* Brod. and *G. Palmeri* Dall. On the eastern shore of America, besides *G. pyramidata* there is another, *G. antillarum* Reeve, described in the Conchologia Iconica in November, 1859, from a single specimen, now in the the British Museum.

"It is not improbable that the latter will prove to be identical, when a sufficient number of specimens are collected for study, with *G. pyramidata;* in which case Reeve's name has priority. All these species of *Glottidia* are probably descended from the same source or ancestral form, and no species have yet been found except in American waters, while on the other hand not a single species of the genus *Lingula*, in the strict sense, is known to occur in America, though all the Glottidiae were originally described as Lingulae. The true Lingulas are almost always attached to a fixed rock or stone, while Glottidia attaches itself, if at all, only when adult, and usually to a very small pebble or bit of shell."

It is hoped that the present account of the structure of this peculiar species of brachiopod will not only throw new light on the structure of Brachiopods in general, but also aid in more closely defining the new genus, the creation of which we owe to Prof. W. H. Dall.

[1] It is now known to occur from the South Florida Keys to Chesapeake Bay.

The Shell of Lingula. (Figs. 1, 2, 3, 4.)

The structure of the shell of Lingula has, so far, been studied only by Gratiolet. This anatomist clearly pointed out the existence of calcareous and horny layers and their alternate arrangement. Gratiolet, however, seemed to be of the opinion that the outermost covering, which is found as a uniform layer over the entire surface of both valves of the shell, including the peduncle, and generally called the *periostracum*, is of exactly the same nature as are the so-called *horny layers* of which the great bulk of the substance of the shell is composed. Both are termed by him *horny*, and no distinction whatever is made between the two in his description. On a closer examination and comparison, the differences in the character of these two structures become, nevertheless, very apparent, and will, I think, be readily recognized. A brief description of the entire shell-structure may, therefore, seem desirable.

Beginning, then, with this outermost covering of the shell, the periostracum, or perhaps better called the *cuticle*, we find it to be a simple, homogeneous, comparatively thin layer or membrane, easily stained by picro-carmine and gold-chloride. Osmic acid gives it more or less of a bronze hue and borax-carmine leaves it entirely unstained. Over the area of the external surface of the shell proper it remains of uniform thickness throughout, but, as it passes from the posterior end of the valves on to the peduncle (over the entire extent of which it is uninterruptedly continued), it becomes markedly thicker and somewhat corrugated, to again assume much less thickness lower down towards the attached end of the peduncle. All around the entire circumference or margin of the shell of both dorsal and ventral valves the cuticle, as it were, overlaps the layers beneath it and forms a rather delicate, incurved margin of the shape of the letter "S" (see Fig. 3) on section, the lower curve of which fits into a cup-shaped impression in the mantle-margin, very near but external to the point where the bristles protrude, and with which it is in organic connection.

In almost all my preparations a certain class of peculiar, round little bodies are seen imbedded in the shell substance, sometimes aggregated in clusters, sometimes arranged in linear series, and at still other times they are irregularly scattered. It is immedi-

ately beneath the cuticle that the largest collections of these bodies are found. (See Fig. 4, *gl.*) They are best seen in sections stained with haematoxyline, by which not only the cuticle but also the horny layers of the shell substance proper are left unstained, while these corpuscles take on a violet color. They may, however, be also very readily observed in picro-carmine specimens.

Although these round little corpuscles, which are perfectly homogeneous bodies and entirely free from nuclei, are generally found aggregated beneath the cuticle, they are also often found irregularly scattered through the substance of the various horny layers forming part of the body of the shell. Shipley has described similar corpuscles in Argiope, and has come to the conclusion that they perform the function of carrying nutriment to the parts over which they are found distributed, believing them to be blood-corpuscles. Having studied these bodies from sections only, it is, of course, impossible to form a correct and positive opinion as to their physiological function, but from purely morphological grounds I regard them as being homologous, if not analogous, with those described as occurring within the organic septa, running vertically in the substance of the shell of the Testicardine Brachiopods.

Immediately adjacent to the cuticle and this layer of homogeneous round corpuscles, we find a rather broad layer of horny substance. (See Fig. 2, *h. l.*) The thickness of the horny layers varies greatly according to the age and the size of the animal; these layers can be beautifully stained with both picro-carmine and borax-carmine, which impart to them a very delicate pinkish hue. Osmic acid and haematoxyline give them a more or less greenish-yellow tint, while gold-chloride stains them a deep cherry brown, and in some cases an intense violet color. The horny substance exhibits sometimes a longitudinally, finely striated appearance, and these longitudinal striae are crossed by others at right angles to them, thus imparting to the whole a very fine, granular character. In most of my preparations the horny layers are homogeneous throughout, showing the above-mentioned round bodies imbedded within them. Internally to this first broad, horny layer, and closely adjoining it, we find a very thin calcareous layer of no peculiar structure and consisting

simply of coarse calcareous granules, which have remained unstained. In this manner the horny and calcareous layers alternate with each other, their number varying according to the size and age of the animal. These alternating layers may be observed equally well in longitudinal and transverse sections. Generally speaking, the horny layers decrease in thickness from without inward, while the calcareous ones slightly increase in thickness in the same direction; in no case, however, have I seen the latter attain a thickness equal to that of the horny layers. All the layers decrease in thickness, as well as in numbers, as we proceed from a point a little posterior to the centre of each valve towards its periphery, where, about the delicate, incurved margin, only a very thin film of the horny substance is left to support the thin cuticle. This thin film joins the supporting layer of the mantle-margin, while the cuticle may be followed a short distance over the epithelial layer of the same, and then gradually passes out of sight and fades away. The layer of ectodermal epithelium, which is very high at the margin of the mantle, is apparently pushed in between periostracum or cuticle and the supporting lamella.

In this manner, as will be found on all good transverse as well as longitudinal sections, all those horny layers composing the body of the valves of the shell become directly continuous with the supporting lamella of the most external layer of the mantle and that of the body-wall respectively. This circumstance quite explains the fact, already observed by Gratiolet, that it is impossible to remove the shell without tearing the external leaflet of the mantle and the body-wall, which together form the lining of both ventral and dorsal valves. Their continuity renders the conclusion almost obvious—namely, that they are identical in structure; in other words, the so-called horny layers of the valves of the shell of Lingula are nothing more nor less than a supporting substance. A further evidence in proof of this fact, if such was necessary, is that both stain alike and with equal intensity. In consequence thereof, it is furthermore not at all unlikely that these horny layers within the valves, from the fact of their being in direct organic connection with the supporting lamella of the mantle and body-walls, represent the homologues of the vertical septa found in the substance of the shells of Testi-

cardine Brachiopods, and certainly the peculiar, roundish bodies scattered through them speak in favor of this view.[1]

The calcareous layers found between the horny layers are probably the result of a secretion on the part of the former, or that of a calcareous degeneration of the ectodermal cells of that part of the mantle and body-wall which is next to the shell.

The cuticle is probably either a changed original larval ectoderm, or has in some way been produced by it.

On the internal surface of the shells of the Lingulas which we have been studying there are found several ridges running in a longitudinal direction. The ventral shell possesses two of these, while the dorsal has only one. The two ridges on the internal surface of the ventral shell are so situated as to divide it longitudinally into three parts, two external areas and a middle one; the ridge on the dorsal shell runs in the median line, and, therefore, when the two valves are in juxtaposition, the latter would point about midway between the two ventral ridges. Longitudinally, they occupy about the posterior two-fifths of the whole length of each valve. The ventral ones converge in a direction toward the peduncle, into which they are, as it were, continued. It is these ridges which show the greatest number of the alternating layers which compose the shell, and to them is also attached the body-wall throughout their whole extent, the breadth of the mantle from about the anterior body-wall increasing in a direction towards the peduncle, the body-cavity decreasing in the same direction in the proportion that the ventral ridges converge.

The growth of the shell and peduncle probably proceeds from apposition to the supporting substance or connective-tissue layer of the body-wall of new layers from within, so that the external layers are the oldest and the internal layers are the youngest.

[1] Van Bemmelen describes certain round little bodies occurring in groups on the surface of the mantle which is next to the valves, defining them as round and sharply outlined little bodies, easily stained by carmine, and as not giving one the impression of regular cells. He noticed the greatest number of them aggregated around the bases of the mantle-papillae. Carpenter also saw these collections of roundish bodies in Testicardine Brachiopods, and thought that they might perhaps be the products of glandular secretion. We have also seen them in large numbers within the sinuses of the mantle.

The Body-Wall, Mantle and Peduncle of Lingula. (Figs. 2, 3, 4, 8, 10, 13, 14, 16.)

The intimate structural relationship which exists between these three organs, the direct continuity of their walls, and the free communication of the cavities which they enclose, seem to make it desirable to describe them together.

The so-called body-wall of Lingula is that portion of it which forms the body-cavity or visceral chamber, within which are contained the stomach, intestines, liver-lobules, genital glands and oviducts, and the various blood-lacunes.

The body-wall may, for convenience of description, be divided into several distinct parts—viz.: an anterior, a posterior, two lateral, a dorsal and a ventral. The dorsal and ventral body-walls are closely adherent to and covered by the valves of the shell, while the remaining parts are comparatively free.

The mantle is but a fold of the body-wall itself, extending from it, in a lateral and anterior direction, around the circumference, along the inner surface of the two valves up to their margins. The outer leaflet of the mantle is attached to all that portion of the inner surface of the valves which is not occupied by the body-cavity. The two mantle-leaflets enclose between them numerous spaces and channels, the most important of which are the so-called mantle-sinuses—four horn-like projections or excavations freely communicating with the body-cavity.

The peduncle may properly be looked upon as a worm-like, backward prolongation of the body-wall and its cavity. Its walls are in the main structurally identical with those of the body and mantle, and the somewhat tubular cavity which they enclose is in open communication with that of the body.

The structural constituents of all three are, first, an outer layer of ectodermal epithelium; second, a middle layer of supporting tissue, variously modified according to situation; and third, an inner layer of lining or peritoneal epithelium.

1. *Ectodermal Layer.*—This, over the greater part of its extent, consists of a single layer of very small cuboidal cells, composed of a homogeneous protoplasm with comparatively large and distinct oval nuclei, which latter stain remarkably well. This character of the ectodermal layer we find especially well preserved all over that surface of the mantle, body-wall and

peduncle which is attached to the valves of the shell, or, in the case of the latter, is covered by the thickened cuticle; it furthermore retains its typical form over the internal surface of the inner mantle-leaflet, extending from the body-wall to the mantle-margin, and also over the lateral body-walls. These cells, however, also present very marked changes in some situations. Thus, for instance, about the margin of the mantle they have become very much larger and elongated; their protoplasm is no longer homogeneous, but presents a very fine, granular appearance; their nuclei are very indistinct, and in some no nuclei whatever can be made out. (See Figs. 4 and 5.) They are oval in shape, and seem to be attached to the underlying supporting tissue by a narrow pedicel.

In specimens which were hardened in picric acid, and afterward stained in borax-carmine, many of the cells appeared enlarged, their nuclei having become very much displaced towards the limiting membrane. Scattered among them were found other cells, peculiar and coarsely granular, oblong and non-nucleated, or without any definable shape; some of them seem to protrude with one extremity from the mantle-margin, hanging loosely into the mantle-chamber; these are apparently amoeboid cells, having originated, not in the ectoderm, but more likely in the mesoderm within the mantle-sinuses. Between these different cells there seems to rise up from the supporting lamella a network of corpusculated fibres (see Fig. 4) with well-stained nuclei. These fibres, which anastomose freely with each other, form a wide-meshed network, which is probably intended to support the rather large cells of the mantle-margin. Over the tip of the latter the cells again become much shorter and narrower, their nuclei occupy a more central position and stain deeply; no granular cells occur here, but the cells are several layers deep. As they go on to form the hair-follicle, they become suddenly flattened, and do not resume their usual characteristic cuboidal form until they have reached the concave surface of the valves of the shell which they are destined to line. Over the remainder of the surface of the mantle, including the bladder-like pouches, called respiratory or branchial pouches, found at the base of the mantle-margin, the ectoderm is composed of a single layer of cuboidal, very regular cells, with slightly oval, well-stained nuclei.

It again differs somewhat from this simple arrangement over the anterior body-wall. Over the lophophore, the bases of the cirrhi, and those portions of the anterior body-wall immediately surrounding the oesophagus, especially over the situation of the central and lateral sub-oesophageal ganglia, the ectodermal covering becomes several layers deep, the cells changing from their ordinary cuboidal form into the more elongated shape; the nuclei becoming at the same time correspondingly smaller, longer and narrower. As it verges off from the anterior to the lateral body-walls, it again assumes its usual character.

We next find it undergoing a rather remarkable change over the posterior body-wall. (See Fig. 13, *p. b. w.*) This deserves a separate mention, and may briefly be described as passing from the attachment of the peduncle to the posterior end of the ventral valve, to the corresponding end of the dorsal valve, thus closing in the body-cavity posteriorly. It is perhaps best seen in longitudinal sagittal sections, taken near the median line, and so directed as to bisect the peduncle as well as the body of the creature. In such sections the posterior wall is seen to pass straight across from the base of the short mantle-margin of the dorsal valve towards the attachment of the peduncle to the ventral valve. Before approaching the latter, it forms a loop by passing straight backward in the direction of the same for a short distance, then turning upon itself and running forward again towards the attachment of the peduncle to the valve. After forming this loop, which, on cross-section (see Fig. 14), is seen to have more the appearance of a semi-circular pouch surrounding the base of the peduncle, the entire body-wall pushes its way into the peduncle, in reality goes to form part of this tubular structure or organ, receiving as it passes backward a very thick covering of cuticle. It is over this portion of the body-wall, and also to a certain extent within the peduncle, that the ectodermal layer of cells again appears in a somewhat modified form. The ordinary cuboidal cells have become distinctly ovoidal and much larger; their contents are granular, the granules consisting of larger and smaller, shining and strongly refracting globules, which do not take on the usual stain, but look yellowish and oily.

Besides these various modifications in the ectoderm, according to different locations, there are also found scattered over the

anterior body-wall and the inner leaflet of the mantle certain large, oval bodies occurring at almost regular intervals, and therefore imparting to these structures a rather studded appearance. These bodies appear usually very uniformly stained with undistinguishable nuclei, and in shape not unlike small taste-buds, with a very minute pore opening to the outside. Their significance is doubtful, and I have been unable to form a positive opinion about them, but think it not unlikely that they are glandular in character.

Immediately beneath the ectoderm we find the supporting substance. In certain situations, however, we have intervening between these two structures the central nervous system, which will be described separately. Certain calcareous plates, or the traces of them, may, very conveniently, be disposed of here. Of these calcareous plates, Van Bemmelen remarks that they are situated immediately beneath the ectodermal covering, and that after the lime has been dissolved out the outline of them remains distinctly visible, showing that each one is surrounded by a membrane, and upon this membrane we also find the cells which formed these plates. (See his Plate IX, Fig. 5.) What V. B. has found in Testicardine Brachiopods with regard to these calcareous plates, is in perfect accord with what we have seen in Lingula. In the latter (See Figs. 2, *vac.* and 15), these plates are more particularly found distributed over the inner leaflet of the mantle and the lateral body-walls. Here, after the lime has been dissolved out, they give rise to a more or less vacuolated layer immediately beneath the ectoderm, in which certain small, spindle-shaped, as well as larger and irregular shaped, finely granular, nucleated cells are not uncommonly found attached to the membrane remaining behind. This condition of things is well seen in those specimens that had been hardened in picric acid.

2. *Supporting Tissue.*—Of all the elementary tissues composing the body of Lingula, this presents perhaps, without exception, the most varied forms. In its ordinary aspect it is a homogeneous layer of tissue either entirely structureless or presenting a very faintly, longitudinally striated appearance, staining deeply with most of the carmine agents. In this form it exists in the so-called horny layers of the valves of the shell, in both leaflets

of the mantle, the dorsal, ventral, and, to a certain extent, also in the lateral and posterior body-walls. In the anterior body-wall, however, and the deeper portions of the lateral body-walls, and also in the peduncle, changes occur which deviate considerably from the ordinary structureless, supporting lamella. Besides the great increase in the thickness which this tissue assumes throughout the anterior body-wall, more especially in those situations in which are found the central nervous system, the oesophageal blood-lacunes, and about the origin of the arms, the most striking difference in structure is in its well-defined cellular elements. (Fig. 20, *c. c.*) The cells are long and delicate, spindle-shaped, nucleated corpuscles, with rather small nuclei, which stain beautifully and deeply. The processes of these cells, usually only two in number, are comparatively broad, very finely granular or homogeneous, and scarcely at all differentiated from the surrounding tissue by staining agents. They usually traverse the entire thickness of the supporting lamella, but sometimes one of them shows two nuclear swellings instead of one, giving it a rather varicose appearance. They are remarkable for their regular arrangement and distribution.

With reference to the lateral body-walls (see Fig. 10), we find, first, a thin layer of ordinary supporting lamella adjoining the ectoderm throughout its whole extent. From this layer, rather abruptly, proceeds a reticulum consisting of supporting tissue, and extending inwardly until it has reached a considerable thickness. The nuclei seen here and there within the tissue remind one of branched connective tissue-corpuscles, the processes of which anastomose with one another to form this network. The rather large meshes of this reticulum are filled with a finely granular, jelly-like material, sperm-material and cross-sections of spermatozoa. The entire mass covers the internal surface of the supporting lamella like a semi-oval pad. We will return to this subject again in speaking of the genital apparatus. Almost the same structural conditions which prevail in the lateral body-walls, but to a much less extent, are found to obtain in the peduncle; here the fibres forming this network are very much finer and their nuclei correspondingly smaller.

The genital bands contained within the mantle-sinuses, also, are essentially like the preceding in their minute structure,

besides their being directly continuous with that portion of the lateral body-walls. The reticulum, however, is much less marked in these bands, having become reduced to a few small fibres, which only in a few instances have been found to anastomose; the greater part of it consists in spermatophores, in all stages of development.

In many places the supporting lamellae of the two mantle-leaflets join one another directly; in others, in which by their separation they have given rise to spaces, the most noticeable of which are the mantle-sinuses, fibrous partitions are seen reaching across from one leaflet to the other. The most important of these are found near the mantle-margin. From a certain point (marked *o* in Fig. 4) of the supporting substance of the outer leaflet several bundles of fibres pass on in two different directions; one set runs towards the margin of the shell, to be inserted at a point corresponding to the extreme end of the delicate, incurved margin of the shell, which here consists only of cuticle and a very thin layer of supporting tissue. This insertion is also external to the point of exit of the bristles from the mantle-margin. The other set of fibres, starting from the same point, becomes attached to the base of the hair-follicle. These two sets of fibres have hitherto been regarded as muscles, but it is evident, both from their structure as well as from their function, that they are nothing more nor less than supporting fibres intended to keep the bristles or hairs in a certain fixed position with relation to the mantle and shell of the animal. The attachment of the most anterior or ascending bundle of fibres to the shell-margin is interesting from the fact that its fibres spread out in a fan-shaped fashion at their point of insertion; in thus spreading out and intercommunicating with the corresponding ends of neighboring bundles and forming a very firm series of arches all around the entire margin of the shell, they are well calculated to keep this margin in a fixed position.

All the so-called mesenteric bands are simply bridges of this substance passing in various directions, but having for their purpose the fixation of the cavities through which they extend, at the same time serving perhaps other purposes.

3. *Peritoneal Epithelium.*—This consists of flattened polygonal cells with small, round, central nuclei; these cells are joined

together edgewise, and form a very thin and delicate membrane which lines the walls of the peri-visceral chamber, the mantle-sinuses and blood-lacunes; this membrane also covers all the viscera and mesenteric bands, and gives rise to blood-vascular spaces and channels within the body-cavity. The changes which peritoneal epithelium undergoes will be considered under the head of "genital apparatus."

The Muscular Apparatus of Lingula.

The anatomy of the muscular system of Lingula has been repeatedly described, with much thoroughness and care, by Vogt, Owen, Hancock, Woodward and Gratiolet. From a careful study, however, of the more minute structure of these muscles, differences have been revealed which would seem to make it in the highest degree doubtful that all those structures described as such, are in reality muscular in character. All the muscles in Lingula are composed of long, parallel fibres, traversing, as a general rule, their entire length, and belong to the variety of *smooth muscle-fibres.* There is no exception to this rule in Lingula, and even the posterior occlusor muscles show no trace of striation, as they have been found to do in some of the Testicardine Brachiopods. All those structures which have been described by Hancock as *parietal muscles*, and which Gratiolet has termed *muscles peaussiets*, to which may further be added the muscles contained within the arms and the peduncle, exhibiting an entirely different structure from ordinary smooth muscle-fibres, we have been led to regard, *not* as muscles, but rather as a *mesenchymatous supporting substance* possessing perhaps a certain amount of elasticity, but lacking the contractility proper to muscular tissue only.

The Vascular System of Lingula. (Figs. 6, 8, 11.)

Both Cuvier and Owen were of the opinion that the pallial sinuses opened directly into the organs designated by them as hearts. Vogt apparently confirmed their opinions by discovering the auricles of these supposed hearts, and also blood-vessels, emanating from the ventricles. Huxley was the first to question the correctness of these supposed facts as ascertained by Cuvier, Owen, Vogt and Hancock, and showed conclusively that there

were no blood-vessels given off from these pseudo-hearts at all, and that, instead of hearts, they were oviducts. Hancock also observed a central organ of circulation in both *Lingula anatina* and *L. affinis*, and found the same to differ but slightly from that of the articulated species.

This heart, which he only saw in a state of contraction, was situated on the posterior slope of the intestine, exactly as in Waldheimia. It is described as being pyriform and rather elongated, with the small end tapering gradually forward. A so-called branchio-systemic vein originates, according to Hancock, in the dorsal mesenteric membrane and communicates, through it and two lateral membranes attached to the oesophagus, with a system of lacunes which surround that tube at its origin much in the same way as in Waldheimia. The vein, as it goes backward, is described as passing between the divisions of the hepatic duct, and is here rather enlarged; it soon assumes the form of a distinct, isolated vein, in which condition it reaches the transverse dorsal ridge of the stomach, from which the gastro-parietal band originates, and at this point opens into the anterior extremity of the heart. The aorta is described as a single trunk leaving the under surface of the large or posterior extremity of the organ, and in this respect differing from that of the articulated species. The aorta is said to pass a considerable way down the straight portion of the intestine before dividing into two lateral stems, which pass outward, and, on reaching the ileo-parietal bands, are again subdivided in the usual way, one branch running forward, the other backward, in connection with these bands. Hancock has traced these branches very much farther, and also speaks of two arterial trunks entering the muscles. According to this author, then, we would have to look, in Lingula, for a closed system of blood-vessels with a central propelling organ of circulation.

Gratiolet, on the contrary, while admitting the existence of the vesicle of Huxley or the heart of Hancock, from his own researches on Lingula, prefers to adhere to the opinion of the older authors, and continues to look upon the oviducts as hearts, regarding the heart of Hancock as only an accessory organ of the circulation. It is to be regretted that Van Bemmelen did not express his views on the vascular system in his article. Shipley, like other more recent observers, was unable to find anything

correspnding to a central circulatory organ and the system of vessels, or the accessory pulsatile vesicles described by Hancock, in Argiope. The blood, he says, is contained in a number of vessels, which run irregularly within the tissues of the body, but which chiefly lie in the mantle and that part of the body-wall lining the shell. Shipley found it impossible to make out distinct walls to these vessels, which appeared to him to be mere slits in the tissues. They were found to be especially numerous at the posterior end of the ventral shell and in the angle formed by the posterior border of the triangular septum and the dorsal shell.

The blood-corpuscles, according to the description given us by Shipley, are large in comparison with the other cells of Argiope; stain deeply around their circumference, and possess no nuclei.

According to Schulgin, Argiope has no central circulating organ nor a closed system of blood-vessels, but a system of peripheral lacunae communicating with the body-cavity. The blood-corpuscles are kept in motion by the ciliated character of the lining peritoneal epithelium.

Prof. Morse seems to have proved the correctness of the latter statement by actual observation in *Lingula pyramidata*, and Brooks, in larval lingulas, has observed blood-corpuscles being constantly driven in and out of the mantle-sinuses through the irregular contractions of the body-wall. Morse compares the circulation going on within the different mesenteric bands to the pseudo-haemal system of authors, and was unable to make out the vesicle on the dorsum of the alimentary canal described by Hancock, and thought by him to be the heart.

Thus, it will be seen that the elaborate circulatory apparatus described by Hancock, in his excellent and comprehensive monograph, has not been found by any subsequent writer on Lingula. Neither the central organ of circulation nor the system of blood-vessels connected with it seem to exist in Lingula, as they were described by him.

Our own observations have been only confirmatory of the views held by Shipley, Schulgin and Morse, and the most careful search after the central propelling organ over the posterior slope of the stomach invariably proved unsuccessful in every new series of transverse sections which was made. There are, however, on both sides of the oesophagus (see Fig. 11, *lac.*) two oblong

tubular organs, generally found crowded with blood-corpuscles and extending from the peri-visceral chamber up to the origin of the arms, into the base of which they apparently empty themselves, communicating by a diverticulum with the sub-oesophageal sinuses. Since, however, they also possess a thin layer of this ubiquitous supporting tissue, they can hardly be supposed to be very contractile and in any way aid in the propulsion of the fluid which they contain. Within the mantle and throughout the anterior body-wall, blood-corpuscles are found in spaces of the supporting substance lined by a layer of peritoneal epithelium, which in my sections did not show any cilia. The accumulations of blood-corpuscles found within the visceral chamber seemed either free, or surrounded by a thin membrane which was composed of peritoneal epithelium only.

There seems to exist still a considerable degree of doubt and uncertainty as regards the true nature of certain cellular elements found floating in the circulating fluids within the body-cavity, and the various other spaces and cavities communicating with the same. In Lingula I was able to make out four different kinds of corpuscles floating in the circulating fluid. There is, first, a small, round, granular corpuscle (Fig. 4, *y*, *o*), more frequently found within the mantle-sinuses and their branches, and sometimes within the large sub-oesophageal blood-lacunes and other parts of the body-cavity. In general appearance these corpuscles do not look very unlike white blood-corpuscles; their contents are granular, their outline is sometimes round, sometimes very irregular, and a nucleus of good size may almost always be found in them; they stain well in borax-carmine, and are to all appearances endowed with amoeboid movement. Their development may easily be traced from the peritoneal epithelium lining the mantle-sinuses, and to a slight extent also from that which lines the mesenchymatous tissue of the lateral body-walls, as well as from the epithelium which covers the viscera.

From a careful study of these interesting bodies, I have been led to regard them as young ova which sooner or later become transformed into fully developed ones. In fact, their development may easily be followed in the mantle-sinuses, where all the stages from peritoneal epithelium to completely developed ova may be found. It may be possible that during the intervals of the

breeding season they remain undeveloped. Secondly, we frequently meet with peculiar, spindle-shaped, or oblong, ovoid, striated bodies already figured and described by Hancock and Gratiolet, and by the former called spermatophores, by the latter regarded as young Lingulas, and by subsequent observers mistaken for blood-corpuscles (Fig. 15, *sp.*); they are found in the same situations as the preceding, and their development from the peritoneal epithelium seems to me an obvious conclusion. The third class of corpuscles have already been described in connection with the structure of the shell; they are the smallest of them all, and are of about the size of the nucleolus in a fully developed ovum; they stain very uniformly in most staining agents, have a sharply defined, round contour, and possess no nuclei; they are found in greatest abundance beneath the cuticle of the shell, and to a less extent also within the mantle-sinuses. The fourth and last class of corpuscles, which are found in greater quantity by far than any of the preceding, are round or slightly ovoidal bodies, of a homogeneous protoplasm, with a sharply defined limiting membrane and a small, usually eccentric, nucleus. Both nucleus and limiting membrane are distinctly stained, while its other contents remain unstained. These are the blood-corpuscles proper.

The Alimentary Canal. (Figs. 6, 7, 9, 11, 12, 20.)

According to Vogt, the intestinal canal of Lingula opens to the exterior and retains about the same width throughout its whole extent. No description of the digestive system is given by either Gratiolet or Van Bemmelen. Shipley, while describing the course and attachment of the different mesenteric bands, says nothing of the histological structure of the different parts of the alimentary canal.

In Argiope, the alimentary tract is said by Schulgin to terminate blindly. The walls of the stomach are described by him as consisting of two layers—namely, an external layer of connective tissue and an internal epithelial layer. The cells of the epithelial layer are said to be narrow, high, and provided with cilia. With reference to these ciliated cells which line the intestinal canal, Schulgin says that each one of them is filled with a *granular protoplasm* which sometimes is found predominating in the upper half of the cells, sometimes in the lower half;

and he has found that when an animal is killed just after taking it out of the sea-water, the granules occupy the more centrally located portion of the cells—that is, the part nearest the lumen of the canal; and that when an animal is killed after having been kept in filtered sea-water for a few days, the granules, on the contrary, occupy the more peripheral portion of the cells. The greatest number of cilia, according to Schulgin, are found during digestion. Schulgin, no doubt, is correct so far as the granular portion of the wall of the intestinal canal is concerned during the intervals and during the act of digestion. So far, however, as the length and the number of cilia are concerned, I must, from my observations on Lingula, question his statement that they are most developed during digestion. In the specimens which were dredged during the month of March, I found the alimentary canal almost completely empty; only a few sparse, large, greenish cells were seen within the liver-lobules. In those specimens, however, which were collected during July and August, the entire intestinal canal, including stomach and liver-lobules, was crowded to distension with diatoms, desmids and other vegetable, and also some mineral, matter. No cilia were observed in such specimens, and the wall of the canal was thin when compared to that of some of my winter specimens. In the latter, the cilia (Figs. 9 and 12) were very long and the granular portion of the wall of the intestine seemed very rich and stained unusually deep, so that I am rather forced to believe that the cilia, during the stage of starvation of the animal, are very much longer and the intestinal canal thicker than during full digestion.

The digestive apparatus of Lingula may be conveniently divided into three parts—viz.: The mouth and oesophagus, the stomach and liver-lobules and the intestinal canal proper. The front portion of the alimentary canal (see Fig. 6) of Lingula, by which is meant the mouth and the oesophagus, forms a horn-like projection through the anterior body-wall. The mouth-opening lies flattened between the ventral valve and the sub-oesophageal blood-lacunes rising up between it and the oesophagus. The opening of the mouth as it looks towards the ventral valve is partially surrounded with a wreath of tentacles, which is, no doubt, calculated to create a current of nutrient material in that direction. From the mouth the alimentary canal pursues a slightly

forward and backward course; then, bending upon itself, it runs straight backward parallel with the long axis of the body of the animal to enter the stomach. The convexity of the canal, then, and not the mouth-opening, forms the most anterior extremity of the alimentary canal. Perhaps the most striking difference in structure between this and the remaining portions of the alimentary canal, is in the fact that the mouth and oesophagus are surrounded on all sides by two layers of strong and dense supporting tissue between which numerous blood-channels wind their way in all directions. One of these layers of supporting substance belongs to the oesophagus proper and the other underlies the general ectodermal covering; the former is continued all over the entire canal and the latter passes into the body-wall.

The largest blood-channels are found to occupy the concavity of the horn, the smallest the dorsum or convexity of the canal; between these two are the lateral ones, intermediate in size. They all communicate with the body-cavity either directly or indirectly, and like it are lined by peritoneal epithelium. Otherwise the mouth and oesophagus do not differ materially in structure from the rest of the alimentary tract. Three layers may generally be distinguished—namely, an outer layer of connective or supporting tissue, a middle layer which consists of granules or very small cells, and an inner or ciliated layer. The middle layer is by far the thickest of them all, and seems to consist of very minute cellular elements which secrete the digestive fluid. Scattered through this layer are found certain cells, shown in Fig. 6, *g. c.*, but better seen in Fig. 20, which I think are apolar ganglion-cells, and may be the remains of some larval sense-organ; the cells are found near the circum-oesophageal commissure only.

The apparent great strength of the tissues surrounding the most anterior extremity of the canal, properly designated as the mouth, and the prominences in the wall itself, seem to imply a certain power for grinding the most solid food-particles before they pass on into the oesophagus.

At a point marked *v* in Fig. 6 will be found a small bundle of supporting fibres passing between the two layers of the supporting tissue. These fibres surround the alimentary canal like a collar in this situation, and are, in transverse sections, found to

be continuous with the several bundles of fibres passing between the large blood-lacunes and the wall of the oesophagus on its ventral side. This valve-like structure may perhaps be looked upon as the dividing line between mouth and oesophagus, and also as having something to do with opening and closing, or at least with rendering the canal wider or narrower, as the case may be. Immediately behind this point the canal becomes considerably wider for a short distance, and just as it enters the body-cavity its lumen becomes very narrow.

Shortly after its entry into the body-cavity, the oesophagus joins the stomach, which is the widest portion of the entire alimentary canal. The stomach gives off several large and broad canals, which repeatedly bifurcate and at last terminate in blind pouches, the so-called liver-lobules. Inasmuch as we have found that the origin of these canals from the stomach occurs with comparatively great regularity in the several series of sections which we have examined with regard to this point, an account of it seems desirable.

The first of these canals is given off on the ventral side; it is short and broad, and divides immediately into two large lateral branches, which in their turn divide and subdivide, and finally terminate in blind pouches. Immediately behind this, the stomach, in cross-sections, presents a rather triangular shape, one of the angles pointing dorsally, the other two ventro-laterally. It is from the dorsal angle that the next canal is given off; it is broad and short, and immediately divides into two branches running at right angles to it and terminating as usual. Next behind this arises another ventral process, much narrower and longer than either of the two preceding ones and nearly reaching the ventral valve before dividing as the rest. The stomach now has assumed a more quadrangular shape in cross-sections, being compressed in a ventro-dorsal direction, convex dorsally and concave ventrally. The fourth diverticulum starts off from the left dorsal angle of the stomach and pursues a direct lateral course towards the left side, while the fifth branch runs from the right dorsal angle and is directed straight towards the right side. In all, five large branches are given off.

The structure of the walls of the stomach is like that of the oesophagus; but the liver-lobules differ very materially from the

ordinary histological structure of the alimentary canal. Fig. 7 is a section representing them, very much magnified. As may be seen, they consist of a thin, loose and supple layer of supporting tissue covered with peritoneal epithelium and often surrounded by numerous blood-corpuscles; an internal layer of well-defined round or polygonal granular cells possessing very distinctly round nuclei and nucleoli. The central lumen of these little pouches is occupied by some very faintly differentiated, round, cellular bodies with long processes of the same material, looking not unlike mucous shreds and probably consisting of secreted fluid coagulated by the reagents used in hardening these tissues and disintegrated cell-structures.

Behind the stomach the alimentary canal assumes again a more rounded form, its walls becoming thicker and appearing less folded (Fig. 9); the part extending from the stomach to the termination of the canal may be termed the intestinal canal proper.

This portion of the alimentary canal passes backward until it has reached the posterior extremity of the body-cavity, always keeping nearer the dorsal than the ventral half; it then forms either a loop or, in some cases also, it simply makes a turn in a forward direction until it reaches the ventral valve passing along its extreme right side, where the latter is joined by the lateral body-wall; it finally becomes very much narrowed, and perforates the body-wall at about the level of the junction of the anterior with the lateral portion, to open to the outside or, in other words, into the mantle-chamber. Throughout its whole length the various mesenteric bands, consisting of supporting tissue, tend to keep it in place. The opening of the anus seems to be valve-like, running for a short distance within the supporting layer of the body-wall before opening to the exterior.

The Nervous System. (Figs. 6, 17, 18, 19.)

It is a fact well recognized by zoologists that the investigation into the structure of the nervous system of the Brachiopoda is accompanied with the greatest difficulty. So far as Lingula is concerned, nothing is as yet known with regard to the histological structure of its nervous apparatus. So able and distinguished an investigator as Hancock confesses that he was not able to even

detect the presence of the sub-oesophageal ganglion in this creature. Owen just mentions the fact that he has seen it; and Vogt, also, I believe, must have noticed it, for he says: "The nervous system is probably situated within the sac formed by the peri-visceral wall near the gullet." The description of the nervous system given by Professor Brooks, in his studies on the development of Lingula, is both significant and interesting in this connection. In certain of the early stages, Brooks describes and figures (see his Figs. 3, 5 and 6) the nervous system as consisting of a commissural ring which encircles the oesophagus at its union with the stomach, carrying one ventral ganglionic enlargement, two lateral ganglia and two dorsal otocysts.

The nervous system of other Brachiopods, however, is much better known than that of Lingula, and Hancock has given us a very complete and detailed account of its anatomy in several Testicardine Brachiopods, especially *Waldheimia australis*. His conclusions were that the nervous collar is situated mainly at the commencement of the alimentary tube, and that with this collar are connected five nervous ganglia, three of which, on account of their superior size, might be assumed to be the principal ganglia.

These nerve-centres, he says, lie amid blood-lacunes, between the two membranes, forming the anterior wall of the peri-visceral cavity immediately below the oesophagus, or rather behind it, on account of the mouth being bent down. He distinctly states that the largest is anterior, the other two form a pair and are lateral and elongated transversely across the median line, and describes the ganglion as having the anterior and posterior margins parallel and the sides sloping inward and backward; it is said to be prolonged into a stout nerve in front, which immediately divides into an upper and a lower trunk.

This description by Hancock of the anatomy of the nervous system in the Brachiopoda has recently been criticised by Van Bemmelen. It is for the sake of comparison that these views have been cited at some length, and for the same reason we will quote those of Van Bemmelen.

Van Bemmelen was perhaps the first to investigate the structure of the Brachiopoda according to modern methods, and the account which he has given us, accompanied as it is with a

number of very finely executed plates, is the most complete of any we possess at present. The descriptions of the anatomy and disposition of the more noticeable ganglia by the two authors vary somewhat.

Hancock, on the one hand, finds five nervous ganglia connected with the nerve-collar; Van Bemmelen, on the other hand, describes them as only two, an inferior and a superior. The lateral ganglia of H. are, according to V. B., only parts of the more centrally situated large sub-oesophageal ganglion with which they were found to be fused, and hence could not be regarded as separate. V. B. further speaks of one superior or supra-oesophageal ganglion situated in the middle line, while H. describes two ganglia in this situation, situated on either side of, but near, the median line. The views of V. B. with regard to the disposition of the different nervous ganglia are, as he says himself, based mainly upon a study of transverse sections.

Shipley describes a sub-oesophageal ganglion situated in the epidermis of that part of the body-wall which is immediately posterior to the base of the tentacles which overhang the mouth, just where the body-wall turns to form the mantle of the ventral shell, as consisting of two parts—namely, an anterior, which is a well-marked elevation formed by a ridge of the homogeneous supporting substance, covered by a layer of nervous cells and fibres, and a posterior portion, which is simply a narrow band of nervous tissue not very conspicuous. The supra-oesophageal ganglion is described by Shipley as elongated, and lying in the ectoderm just anterior to the base of the lip which overshadows the mouth; this ganglion is said to be very small in comparison with the sub-oesophageal.

Finally, Schulgin, studying the nervous system from sections as well as teased preparations, locates the sub-oesophageal ganglion deep under the mouth, at a point where the ileo-parietal joins the ventral band beneath the lower margin of the disc of tentacles. Behind (dorsally ?) he finds two small lateral ganglions, directly connected with the lower ganglion by fine nerve-fibres. The dorsal connection between these two he was unable to trace, but suspects that they are so connected as to complete the circum-oesophageal nerve-ring, on account of

their sending off fine nerve-fibres which tend to meet in the middle line. Schulgin then speaks of a sense-organ, which he locates in the anterior body-wall, just where the great sub-oesophageal ganglion of authors is usually situated.

From this brief review of the accounts of the nervous system, we may readily see that discrepancies still exist. On closer examination, however, they will be found unimportant. Nevertheless, from our own investigation into the nervous system of Lingula, we have been led to adhere to the number and division of the nervous ganglia originally put forth by Hancock for *Waldheimia australis.* There are in Lingula five distinct nervous ganglia connected with the circum-oesophageal commissure. These are sufficiently separate and distinct from each other so as to justify their being accepted as different and as needing separate description. The number of the ganglia in Lingula, therefore, agrees with that found in Waldheimia by Hancock. These five ganglia may, from their respective situations in this instance, be described as the *great central sub-oesophageal*, the two *ventro-lateral*, and the two *dorso-lateral* or *supra-oesophageal* ganglia. The first-named is also the largest; the second comes next in size, and the third is the smallest of them all. All the ganglia are enclosed between a layer of ectodermal epithelium, forming their outer covering, and consisting of one or more layers of cells, and a layer of supporting substance, of varying thickness, forming the floor.

The great central sub-oesophageal ganglion occupies the entire extent of that portion of the anterior body-wall which is situated between the oesophageal blood-lacunes and the mantle lining the ventral valve. In shape it is plano-convex, the convexity being anteriorly. At the dorso-lateral angle on either side, the ganglion is connected by a thick commissure with the two ventro-lateral ganglia. As may be seen, this account regarding the extent and location of this ganglion agrees perfectly with that given by all the authors above quoted, with the exception of one. Schulgin describes a condition of things as existing in *Argiope Kowalewskii* which is somewhat remarkable, if not unique. In about the situation where the sub-oesophageal ganglion is found in all the other Testicardine Brachiopods, as well as in Lingula, he locates two sense-organs. (See his Figs. 17 and 18.) On page 137 of

his article he says: "Argiope possesses not far from the mouth a very characteristic accumulation of cells upon the integument, which plays the part of a sense-organ. This organ consists of two parallel, longitudinal heaps of cells, one of which, nearest the mouth, containing specific, and the other, farthest away from the mouth, containing epithelial, cells." The former is spoken of as being in direct connection with the central or sub-oesophageal ganglion, and as not being an organ of sight on account of the absence of pigment. The central oesophageal ganglion—which, therefore, must be very small—he locates much nearer the oesophagus and the tentacular disc than is ordinarily the case in other instances. No such sense-organ as Schulgin describes in Argiope exists in the Lingulas we have been studying.

The next ganglia, those which we have designated as *ventro-lateral*, are two in number. They are situated close by the sides of the great oesophageal blood-lacunes, the outer boundaries of which they cover to a certain extent, being, of course, separated from them by a thick layer of a homogeneous supporting substance. Their situation with relation to the oesophagus is ventral, and to the great oesophageal ganglion it is dorso-lateral. Their shape may be said to be semi-oval, the convexity pointing to the right and left sides respectively, and the plain surface towards the lacunes. Both ganglia are connected with each other by a strong transverse commissure running straight across from side to side on the ventral aspect of the lacunes, or on the dorsal side of the great sub-oesophageal ganglion—that is, between the two; their outer ventral extremity is joined to the central sub-oesophageal ganglion at its dorso-lateral angle; their dorsal extremities give off the dorsal pallial nerves and afterward form the continuation of the commissure. The commissure passes on towards the posterior aspect of the roots of the arms, and here it divides into two bands, one of which passes around the oesophagus on the ventral aspect of the arm-roots, and the other over the dorsal aspect of them; from both these bands numerous fine fibres are given off to the surrounding tissues; they finally join and fuse with the supra-oesophageal ganglia.

The *supra-oesophageal* ganglia are two in number; they are

situated over the anterior aspect of the roots of the arms, extending to the sides of the most anterior and curved extremity of the alimentary tube, over which a commissure passes connecting the two. The somewhat triangular space in this situation is thus completely filled out. The nerves which these ganglia give off to the arms, and which pass off from them in a lateral direction, are exceedingly rich and unusually large and capable of being traced for some distance.

With reference to the minute histological structure of the nervous system in Lingula, a general uniformity obtains. The nerve and ganglion-cells, as indeed most of the other cells in this creature, are all very small, and high powers of the microscope are, therefore, to be employed, in order to see and recognize them. A good No. 9 Hartnack obj. with a No. 3 eye-piece is usually sufficient for the purpose. With such powers, the structure of the nervous system will be best revealed in specimens carefully preserved with picric acid and stained in borax-carmine. In such specimens the larger ganglia are found to consist of multipolar ganglion-cells, with broad and long processes anastomosing with each other, and forming a thick and dense network. These cells vary very much in size and shape, and possess usually round, well-stained nuclei and nucleoli; their contents are finely granular, but not uniformly so; sometimes one portion of a cell seems granular, the other portion homogeneous, and so on. Every large ganglion-cell usually gives off one long process, which passes towards the supporting lamella, and there is lost in the general felty network of the numerous fibres congregating in that situation. These processes are best seen in dorso-ventral longitudinal sections through the great sub-oesophageal ganglion (Fig. 17), and are much less noticeable in the other ganglia.

So far as the course and distribution of the different nerves are concerned, they have been so accurately described by V. B. that there remains nothing new for us to add in respect to them. We may, however, say that in all essential points the distribution and course of the various nerve-trunks in Lingula agree perfectly with the description given us by V. B. of Testicardine Brachiopods.

Before leaving the nervous system, mention must be made of certain cells the nature of which seems to be as yet doubtful.

In Figs. 19 and 20 will be found certain well-defined cells, strongly granular, with distinct nuclei and nucleoli, imbedded in the wall of that portion of the oesophagus which is surrounded by the nerve-ring and the ganglia in connection with it, which cells may perhaps be regarded with a certain show of reason as apolar ganglion-cells; it is also not quite improbable that they are the remains of some larval sense-organ, rather than salivary corpuscles.

Schulgin also mentions certain sensory cells which he found between ectoderm cells in some situations. I have not been able to convince myself with any degree of certainty of their existence, unless some of the peculiar inter- or sub-ectodermal cells shown in Figs. 2, 4 and 5, are sensory in nature, which might possibly be the case.

The Generative Organs. (Figs. 4, 10, 15 and 16.)

The description of the genital apparatus of Lingula may be divided into two parts—namely: 1. That of the *genital glands*, which are the organs producing the ova and spermatozoa respectively; and 2. That of the *oviducts* or *segmental organs* (Morse), which conduct the spermatozoa and ova into the pallial chamber and thence into the sea-water.

The segmental organs have received by far the greatest amount of attention from the investigators of this class of animals, while the genital glands, more especially those which produce the spermatozoa, are as yet but very imperfectly understood; and one of the main objects of this paper is to throw some additional light on this subject, if possible.

Before proceeding to a more minute description of the genital organs as they exist in the creature we have been studying, it will be necessary to take a look over the somewhat extensive literature of this field of research, in order to realize the present state of our knowledge.

Cuvier, Owen and Vogt, the oldest writers on Brachiopods, as has been mentioned before, had no hesitation whatever in pronouncing as hearts what we now know to be oviducts, or perhaps what we might more correctly term, with Morse, segmental organs; and even Gratiolet, who wrote some time after Hancock

had published his views on the subject, still continued to adhere to the opinions of these older writers.

It is more especially Hancock's comprehensive article on the anatomy of Brachiopods which claims our attention for a moment. The views expressed by him very justly became the most generally accepted ones of his day, and it is perhaps but fair to state, in general terms, that even at the present day most of his views, advanced nearly thirty years ago, cannot be overthrown.

Of the genitalia of *Waldheimia australis*, Hancock says that they are formed of thick bands somewhat convoluted and branched; that they are of a yellow color and are thrust into the trunks and main branches of the great pallial sinuses. There are four of these bands, two in each lobe; those on the dorsal lobe are single and occupy the two outer or lateral sinuses, extending from behind the occlusor muscles to within a short distance of the anterior margin of the mantle.

Their posterior extremities reach the peri-visceral chamber. The ventral pair extend as far forward as the dorsal, and are double—that is, each forms a loop the free extremities of which pass into the outer and inner sinuses of the same side; the looped portions lie within the peri-visceral chamber at the sides below the oviducts.

These genital bands, according to Hancock, are attached to the inner lamina of the mantle throughout their whole extent by a membrane which, originating in the lamina, passes into a groove extending along the under surface of this genital band. The genital or pallial artery is said to run along the edge of this membrane, and has the reproductive organs developed around it. On closer inspection he finds that these organs are in reality developed between the two membranes which, it will be afterwards seen, compose the inner lamina of the mantle, and bulging out the interior of these, become suspended, as it were, in the pallial sinuses.

Somewhat farther on in the text, Hancock says that the genitalia are very perceptibly composed of two elements—the yellow, ovigerous substance which forms the chief mass, and a red material, for the most part distributed over the surface of this organ. When, he remarks, the organ is in a low state of development, this red matter forms a narrow, irregular cord, which runs

along the side of the band and is occasionally spread over the surface of it in spots and blotches. When the ova are fully developed, this substance may still be seen as small specks on the surface and throughout the mass.

This red matter, Hancock states, may prove to be the testis, and is made up of large, irregular cells inclining to oval, variable in size, and without any apparent nucleus.

This whole account refers mainly to *Waldheimia australis*.

This description of the generative glands in *Waldheimia australis*, a Testicardine Brachiopod given by Hancock, is in almost perfect accord with what we have found with regard to the genital glands in the Ecardine Brachiopod Lingula. Indeed, with one exception, they are identical. This exception is, that in the species of Lingula we have been studying, the genital bands contained within the mantle-sinuses are attached to the outer leaflet and have no genital artery, instead of their being attached to the inner leaflet of the mantle and provided with a genital artery, as is the case in Waldheimia.

Nevertheless, Hancock states from Lingula, of which he studied two species—namely, *L. anatina* and *L. affinis*—that *the reproductive organs are withdrawn altogether from the mantle-sinuses and are placed in the visceral chamber*, as they are also stated to be in Discina. Hancock describes the reproductive organs of Lingula as being very bulky, and as occupying a very large portion of the visceral chamber; they lie, he says, for the most part, behind the liver and surround the alimentary tube, forming four irregularly branched and lobed masses, two above and two below the tube, which he designates as the dorsal and ventral lobes respectively. The ovaries themselves are, according to Hancock, suspended by the parietal bands and their reflected portions, which agrees perfectly with the condition of things as they exist in *Lingula pyramidata*, so far, of course, as that portion of the generative glands is concerned which is contained within the visceral chamber.

It becomes now necessary to examine Hancock's views as to a certain reddish-yellow mass or substance which he found covering the surface of the ovaries, and which he supposed to be the testis. This substance, he remarks, assumes the form of a dendritic or branched organ as it spreads over the surface of the ovarian masses.

Over the dorsal ovaries he describes this organ as passing from behind forward in two lateral divisions, on the ventral ones in three, two lateral and one median.

He also observed, when attempting to remove the membrane forming the dorsal and ventral walls of the visceral chamber, that those dendritic organs came away with it. He was *originally* induced to believe that they were organically connected with it, but further experience, he says, convinced him of the fact that they were really a portion of the genital mass—that is, of the ovarian masses contained within the peri-visceral chamber—and had nothing to do with the body-walls. Their adhering to and coming away with these walls, he thought was explained by the pressure which was exerted on these masses by the closure of the valves.

Nevertheless, if Hancock had adhered to his original impression, and had followed it up more carefully than he did, it would, in our opinion, have led him to the true origin of these dendritic masses, which, as we will learn later on, are really organically connected with the body-walls.

That this so-called dendritic or branched organ of which Hancock speaks, was really the testicular mass, may be inferred with certainty from his own microscopical examination, which proved it to be composed of irregular cells, somewhat elliptical in form, and closely resembling the reddish substance observed in connection with the ovaries of Waldheimia. The cells in Lingula, however, appeared to him to present different stages of development, varying much in size and shape and being filled with numerous hair-like bodies resembling spermatozoa. A glance at Fig. 16, *sp.*, will, perhaps, answer the description of the bodies he describes.

Whether or no Hancock discovered the true origin of these bodies, it is sufficient for our present purposes to know that he came nearer to it than any of his successors, and that he did recognize in them their true nature, which is that they formed the male element of generation. His conclusion, therefore, that the sexes in all the Brachiopods were united, was, of course, natural enough.

Fig. 10 is a small part of a transverse section through the lateral body-wall, showing the origin and true seat of develop-

ment of the spermatophores and the epithelium covering them on their internal surfaces. All over the internal surfaces of both lateral body-walls the spermatophores are densely crowded together, forming a very thick layer, which extends far into the peri-visceral chamber, coming into close contact with the mesenteric bandlets from which the ova are principally developed, and which, therefore, the spermatophores seem to cover. Figs. 15 and 16 show their development from the genital ridges which are contained within the mantle-sinuses and their branches.

The intimate structure of the genital ridges, their loops, which hang in the peri-visceral chamber, and that of the lateral, and, to a certain extent, also of the dorsal and ventral body-walls, is in the main identical. These structures are all in direct continuity with each other, and their structural differences, which are slight, will again engage our attention at the end of this chapter.

Very different from the views of Hancock are those advanced by Gratiolet, who believes that the peculiar bodies described and named by Hancock *spermatophores*, are not spermatophores, but *young Lingulas.* Gratiolet believes Lingula to be an hermaphrodite animal, in which, however, hermaphroditism is not simultaneous, but rather successive; that is to say, the animal is first a male, filling the visceral cavity with a spermatic fecundating fluid to fructify the ova, which are to be developed subsequently, somewhat after the fashion of Davaine's theory of the fructification of the oyster. The structures which develop the ova also develop the spermatophores, according to Gratiolet.

Shipley, in his article on Argiope, makes the following remark with regard to the sexual apparatus—viz.: "Although I have not been able to find a male Argiope, yet I have no doubt that this genus, like the other members of its class, is dioecious. In those Argiopes which I have examined I find no trace of a testis, and in the allied genera, Lacaze-Duthiers describes a male generative gland in a position similar to that occupied by the ovary in Argiope, and I have myself seen the testis of Megerlea in the same position."

Strangely enough, Schulgin, one of the latest investigators of Brachiopods, likewise states that in all the species of Argiope examined by him (which include a large number) he was unable to find a single male specimen, but, nevertheless, expresses himself as very decidedly in favor of the bisexuality of Argiope.

The question might be asked, Could both Shipley and Schulgin perhaps have overlooked the existence of spermatophores, and misinterpreted the structures from which they are developed?

With reference to this point, Prof. Morse says that he believes that in all the Brachiopoda the sexes will be found separate, and that in Lingula the spermaries occur in the peri-visceral cavity in masses like the ovaries. Having studied them alive, Morse continues, it was found that while in some individuals the ovarian masses nearly filled the peri-visceral cavity, in others the spermaries occupied similar positions. According to Morse, Lingula and Discina are identical in this respect.

Van Bemmelen also believes in the sexes being separate, at least in Testicardine Brachiopods, while Oscar Schmidt holds that they are united in the same individual. It is clear, then, that the weight of evidence is in favor of the bisexuality of the group, and all the more modern authors are almost unanimous on this point. It was, therefore, only after considerable hesitation that I was forced, by the evidence before me, to believe in the fact that, so far at least as Lingula is concerned, the sexes are united within the same individual.

No specialized male organs of generation have so far been described as occurring in Lingula; but it was thought that wherever, in the female, ova were developed, in the male, spermatozoa were found to develop; and even Hancock was of that opinion, although he saw both develop side by side within the same individual.

Hancock's views are still correct, so far as the genital ridges within the mantle-sinuses are concerned. Within these, according to our interpretation, both ova and spermatophores develop side by side; it is, however, different within the peri-visceral chamber. Here the ova are confined principally to the mesenteric bands and their reflected portions—in other words, occupy a more central position with relation to the animal—while the spermatophores occupy the peripheral walls of the visceral chamber—in fact, are almost exclusively developed from the peritoneal epithelium covering (in many layers and much modified) the lateral body-walls and to a slight extent also the dorsal and ventral.

While, then, in our opinion, Lingula is an hermaphrodite

animal, it is nevertheless rare to find both ova and spermatozoa present in equal proportions and equally developed within the same individual. In those individuals in which, for instance, the male elements largely preponderate, fully developed ova are sometimes very few, and may even be found entirely confined to the mantle-sinuses, so that, on a superficial examination, they might be entirely overlooked. A more careful examination of an entire series of sections, however, will invariably result in finding both male and female organs of generation within the same individual.

As already mentioned, the principal seat of development of the spermatophores is the lateral body-wall. This arrangement seems to be in perfect harmony with the close apposition in some individuals of the cup-shaped internal extremity of the segmental organs to the lateral body-walls, which, so far, has remained unexplained. One of Gratiolet's objections to the view that these organs are oviducts instead of hearts, was this very circumstance. He said if they are oviducts, intended to carry the ova from the peri-visceral chamber to the exterior, their internal openings ought to be directed towards the eggstocks; meanwhile, they are closely applied to the lateral body-walls. But we know also that the internal openings of the segmental organs are not always in this position, but occupy places which would materially favor the passage of ova into them. We would, therefore, consider them as movable organs intended to take up spermatozoa or ova and carry them into the mantle-chamber at certain intervals; and in accordance with this double function the relative position of their internal openings changes: at one time it will be found snugly applied to the lateral body-walls, and then spermatophores may be seen within the oviducts; at another, their ciliated inner extremity will point directly backward towards the most posterior portion of the visceral chamber, into which fully developed ova usually drop, and under these circumstances ova may be detected within the oviducts. Having never seen either ova or spermatophores within the same oviduct, it is not to be supposed that fructification takes place inside of the animal, but rather that this occurs in the mantle-chamber or in the sea-water.

With reference to the mode of development of the ova—so far, of course, as this may be studied from sections only—it appears

to be about as follows: To begin with, we think there can now hardly be any doubt as to the ova springing directly from the cells composing the peritoneal lining membrane. The cells composing this structure are, under normal conditions, small, round or polygonal cells joined together edge to edge; they possess a well-defined and usually round nucleus, which lies imbedded within the homogeneous cell-substance. Over the mesenteric bands and their reflected portions, where ova are developed, these cells at first become roundish, lose their transparency and take on a granular character. The nucleus now seems somewhat obscured, but as development proceeds it reappears, and is now much larger than it was before. During the intervals of the breeding season the ova in this stage of development may become detached and wander about, growing larger, however, all the time, so that ova in almost all stages of development may be found floating around everywhere. Finally, the granular contents become differentiated into the yolk and the germinal vesicle is formed from the nucleus, and so is also the germinal spot. During the breeding season almost all these different stages may be studied on one single branch of mesenteric filament, the ripest ova being always found near its end and farthest away from the main stem.

The development of the spermatophores, which also takes place from peritoneal epithelium, is somewhat different. Here the nucleus seems to be the all-important element. This at first becomes slightly oval and elongated, being surrounded by a zone of clear, transparent, structureless protoplasm. The nucleus now assumes a very finely longitudinal, striated appearance, grows rapidly, until it finally entirely fills its surrounding envelope, which it seems to burst open, when it becomes a fully developed spermatophore. Fig. 16 will illustrate these points. The development of the ova is, perhaps, best followed by examining Fig. 21.

The oviducts having been repeatedly and very accurately pictured and described by most authors on the anatomy and structure of Brachiopods, it would seem superfluous to say anything more about them here, since my studies have only confirmed the correctness of the descriptions given us by the more modern authors on the subject.

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EXPLANATION OF PLATES.

PLATE XIV.

FIG. 1.—Represents a transverse section of shell and mantle from a point about midway between margin of mantle and anterior body-wall; *h. l.*, horny layer; *c. l.*, calcareous layer of shell with *cu.*, cuticle; notice the peculiar round, little globules beneath the cuticle; *ec.*, ectodermal epithelium; *m. s.*, mantle-sinus lined with *p. e.*, peritoneal epithelium; *g. r.*, generative ridge, × piece of shell and cuticle broken off. Magnif. Ocul. iii, Objective vi.

FIG. 2.—Transverse section through mantle and shell, on larger scale than preceding; letters same as in Fig. 1, showing peculiar so-called calcareous corpuscles, *c. c.*, and the vacuoles left between ectoderm and supporting lamella after treatment with picric acid, *vac.* Magn. Oc. iii, Obj. 9, Hart.

FIG. 3.—Section through margin of shell and mantle; *sh.*, shell, *cu.*, cuticle; *ec.*, ectodermal layer next to shell; *h.*, hair; *t.*, tip of margin of mantle. Magn. Oc. iii, Obj. 9.

FIG. 4.—Transverse section through margin of mantle, shell and mantle-sinus; *cu.*, cuticle; *h.*, hair; *sh.*, body of shell diagrammatically represented; *s. f.*, supporting fibres; *ec.*, ectodermal layer lining shell; *y. o.*, young ova; *g. r.*, generative ridge contained within *m. s.*, mantle-sinus; *sp.*, spermatophores; *m. m.*, mantle-margin; *ec.*, ectodermal layer; peritoneal epithelium lining the entire mantle-sinus, except over generative ridge, where it is much modified; *pl.*, plexus of corpusculated supporting fibres or sensory cells of Schulgin.

FIG. 5.—Section through part of mantle-margin; gold preparation; *s. t.*, supporting tissue and hair diagrammatically represented; mantle-margin showing large granular cells, *gr. c.*, stained a very dark, brownish-red; *f. gr. c.*, finely granular cells almost unstained; ?, peculiar, spindle-shaped, elongated cells which may be either supporting fibres or perhaps also the sensory cells of Schulgin, to which they seem to correspond both in situation and appearance. Magn. Oc. iii, Obj. 9.

FIG. 6.—Dorso-ventral longitudinal section from borax-carmine specimen; *lac.*, space hollowed out of the supporting tissue, various sizes and all lined by peritoneal epithelium; *v.*, bundle of supporting fibres forming a sort of partition and extending laterally and horizontally to a considerable distance around that portion of the oesophagus, probably serving as a valve of some kind; *oe.*, wall of oesophagus; *cav.*, cavity of oesophagus; *cil.*, ciliated portion of oesophagus; *l. l.*, liver-lobule; *n. s.*, section of part of central suboesophageal ganglion; *bl.*, blood-corpuscles; *t.*, section of one of the tentacles projecting from the lower lip, *l. p.; s. t.*, bundles of supporting fibres running between two layers of supporting tissue, and probably subserving the same function as *v.; u. l.*, upper lip; *g. c.*, peculiar large cells, probably apolar ganglion-cells. Magn. Oc. iv, Obj. 6.

PLATE XV.

FIG. 7.—Section of liver-lobule, showing thin outer coat of loose supporting tissue, *st.*, with peritoneal epithelium, *p. e.;* one or two layers of large, round or polygonal granular cells next to supporting

layer, *l. g. c.*, and very large and finely granular cells, *m. c.*, in the centre, with processes looking like mucous shreds. Magn. Oc. iii, Obj. 9.

FIG. 8.—Dextro-sinistral longitudinal section of mantle intended to show the structure of the papillae, which are filled with blood-corpuscles. Magn. Oc. iii, Obj. 6.

FIG. 9.—Transverse section of intestine below stomach, showing an outer coat of loose connective or supporting tissue, in which numerous blood-corpuscles are generally found, not represented in the drawing; *w.*, wall; *cil.*, ciliated internal layer. Magn. Oc. iii, Obj. 6.

FIG. 10.—Transverse section through lateral body-wall; *ec.*, ectodermal covering; *vac.*, vacuoles; *l. c.*, lime-cells; *s. l.*, supporting lamella; *p. e.*, modified peritoneal epithelium; *r. sp.*, cross-sections of ripe spermatophores; *sp.*, spermatophores. Magn. Oc. iii, Obj. 9.

FIG. 11.—Transverse section of oesophagus, intended to show the two lateral blood-channels, *lac.*, extending between the roots of the arms and the peri-visceral chamber, and also communicating with the sub-oesophageal blood-lacunes; they are always filled with blood-corpuscles; *s. f.*, strong bands of supporting lamella separating the ventral blood-lacunes; *oe.*, oesophagus. Magn. Oc. iii, Obj. 3.

Fig. 12.—Longitudinal section through intestinal canal below stomach; *p. e.*, very dense layer of peritoneal epithelium; *st.*, supple layer of supporting lamella; *w.*, wall of intestine; *cil.*, ciliated internal layer of same.

FIG. 13.—Dorso-ventral longitudinal section taken through the median line, showing *p. b. w.*, posterior body-wall with peculiarly modified granular ectodermal cells; *pch.*, semicircular pouch or margin surrounding base of peduncle, *ped.*; *cu.*, thickened cuticle of peduncle; *st.*, network of fine supporting or connective tissue fibres; *p. o. m.*, posterior occlusor muscle. Magn. Oc. iii, Obj. 6.

PLATE XVI.

FIG. 14.—Transverse section from near origin of peduncle from posterior end of ventral valve; *cu.*, the thickened cuticle; *l. bl.*, large blood-vessel; *cp.*, cavity of the semicircular pouch seen in longitudinal section in Fig. 12; *p. b. w.*, modified ectoderm cells of posterior body-wall; *p. o. m.*, diagrammatic representation of attachment of posterior occlusor muscle. Magn. Oc. iii, Obj. 7.

FIG. 15.—Transverse section of portion of mantle, with shell through mantle-sinus showing genital ridge, *g. r.*, filled with

numerous granules of sperm-material; *s. p.*, spermatophores; *h. l.*, horny layers diagrammatically represented; *c. l.*, calcareous layer of shell; *l. c.*, lime-cells; *m. e.*, beginning modification of peritoneal epithelium; *ec.*, general ectodermal covering; *sh.*, shell. Magn. Oc. iii, Obj. 7.

FIG. 16.—Section through mantle in situation of branchial pouch at base of mantle-margin, showing generative ridge and contents; borax-carmine specimen; *ec.*, general ectoderm; *l. c.*, lime-cells; *p. e.*, peritoneal epithelium; *y. o.*, young ova; *sp.*, spermatophores of various sizes, × the point at which peritoneal epithelium begins to become changed. Magn. Oc. ii, Obj. 4.

Fig. 17.—Dorso-ventral longitudinal section through central sub-oesophageal ganglion, showing the ventral half of the ganglion; *ec.*, general ectodermal covering, the cells here running several layers deep; *l. g. c.*, very large multipolar ganglion-cells; *pr.*, peculiar wavy processes emanating from these ganglionic bodies and pursuing an antero-posterior course, finally splitting up into finer branches, and becoming lost in the thick and dense, felty network of fibres bordering on the supporting lamella; *s. t.*, supporting tissue very much thickened. Magn. Oc. iii, Obj. 9.

FIG. 18.—Transverse section through about the centre of the lateral sub-oesophageal ganglion; *ec.*, general ectodermal covering; *l. g. c.*, large multipolar ganglion-cells; *s. t.*, supporting tissue very much thickened in this situation.

FIG. 19.—Section through supra-oesophageal nerve-ganglion and portion of the most anterior extremity of alimentary canal; *g. c.*, probably apolar ganglion-cells imbedded in the wall of the oesophagus; *s. oe. g.*, supra-oesophageal ganglion, in structure essentially the same as the other two, but with smaller cells; separated from the oesophagus by a thick layer of loose supporting tissue, *s. t.*; *ec.*, general ectodermal covering; *p. e.*, peritoneal epithelium of large sub-oesophageal blood-lacune. Borax-carmine spec. Magn. Oc. iii, Obj. 7.

PLATE XVII.

FIG. 20.—Transverse section of anterior extremity of oesophagus and gullet, taken at about the level, marked *v.* in Fig. 6; the drawing is made from the extreme lateral portion of the transverse slit leading into the mouth; *ec.*, general ectodermal covering; *lac.*, blood-lacunes; *a. l.*, extreme lateral portion of anterior lip of mouth; *sp.*, space left between *a. l.*, anterior lip, and *p. l.*, posterior wall of mouth or gullet, and showing cilia; *g. c.*, peculiar large cells with thick and numerous

granules, well-defined and usually eccentric nuclei and nucleoli, probably representing apolar ganglion-cells; *c. c.*, spindle-shaped cells, possessing one or more nuclei and imbedded in *s. t.*, which is to represent the homogeneous supporting substance in this situation; *lac.*, ordinary blood-lacunes. Magnif. Oc. ii, Obj. 9.

FIG. 21.—From a dorso-ventral longitudinal section, showing bands of supporting tissue covered with peritoneal epithelium undergoing modification and in the process of forming ova; *m. p.*, modified peritoneal epithelium; *f. d. o.*, fully developed ova, ready to drop off, always to be found at the most dependent portion of these genital bands suspended from the oviducts and mesenteric bands. Picrocarmine specimen. Magn. Oc. iii, Obj. 9.

OBSERVATIONS UPON THE BLOOD OF LIMULUS POLYPHEMUS, CALLINECTES HASTATUS AND A SPECIES OF HOLOTHURIAN. By W. H. HOWELL, Ph. D., Associate in Biology, Johns Hopkins University. With Pl. XVIII.

The chemical and microscopical study of the blood of *Limulus Polyphemus* and *Callinectes hastatus*, the results of which are given in the following paper, was undertaken by the author partly with the hope that it might throw some light on the supposed relationship of the Limulus to the crustaceans.

There seems to be no good reason why, to a certain extent, there should not exist in closely related animals, having the same general habits of life, a fundamental similarity in the chemical composition of the blood—what might be called a homology of chemical composition, comparable to the homologies of anatomical structure, which are of so much importance in determining relationships; though different habits of life in animals originally sprung from a common stock, or similar habits of life in animals not closely related, might very well bring about, in the first case, changes in the composition and properties of the blood, or, in the second case, produce a similarity not caused by community of origin. It is possible, though facts are wanting to give the idea any stronger claim to notice, that in the blood, for instance, chemical substances, albumens, that no longer possessed important functions in the nutrition of the animal, might nevertheless persist in the blood as remnants of a former mode of life, analogous to the rudiments of anatomical structure, and, if correctly understood, might, as well as these, give useful information with regard to the true affinities of the animal.

The main purpose, however, that was held in mind during the investigation, was that the results might contribute to a better understanding of the chemical and microscopical phenomena of coagulation in mammalian blood. There is every reason to

believe that the process of coagulation among these lower animals is a simpler act, and offers easier conditions for study than we have among the higher forms. A knowledge of the process here must certainly throw some light on the same phenomenon among the higher vertebrates. Very little can be expected in this direction from the study of two or three isolated forms, and I hope in the future, as occasion offers, to add to the facts here given the result of investigations on the blood of other invertebrates. The results here given were obtained during a stay of two months at the Marine Laboratory of the Johns Hopkins University in the summer of 1885. I desire to express my thanks to the director of the laboratory, Dr. W. K. Brooks, for his kindness in placing facilities for work at my disposal.

In a late number of the *Journal of Physiology*, Vol. VI, No. 6, there is a paper by W. D. Halliburton, "On the Blood of Decapod Crustacea," which has appeared since my work was completed. In some points Halliburton's results on the crustacea coincide with those obtained by me from Callinectes, and in some points we differ; but, inasmuch as my work was done chiefly upon the Limulus, while Halliburton appears to have had only incomplete opportunities for studying this animal, the publication of my investigations will still add some new facts to our knowledge of the chemistry of invertebrate blood.

I.—LIMULUS POLYPHEMUS.

Limulus occurs in large numbers on our coast during the summer months. On account of the large quantity of blood it contains, and the ease with which this blood can be obtained, it makes a very convenient animal for study. The usual method of getting blood was to puncture the heart through the soft integument joining the abdomen to the cephalo-thorax, and allow the blood to spurt directly into a receptacle. In a few cases a hole was trephined through the carapace, and a cannula inserted into one of the aortic trunks springing from the anterior end of the heart. The blood was pumped directly through the cannula and the rubber tubing connected with it into the receiving vessel. The blood obtained by this method did not seem to be any purer than that obtained by simply puncturing the heart, so the method was soon abandoned.

Investigations of the blood of Limulus have been made by Genth,[3] who concerned himself chiefly with the ash of the blood, demonstrating in it the presence of copper, and more recently by Gotch and Laws,[3] and also Halliburton.[1] The work of Gotch and Laws, as far as the organic constituents of the blood are concerned, is very incomplete and in some points erroneous. The work of Halliburton will be spoken of farther on in the course of this paper.

The blood of the Limulus as it escapes from the heart is at first of a milky white color, but soon changes on exposure to the air to a dirty bluish white. Within a few seconds after being shed the blood begins to clot. The coagulation of the blood, as far as my experience goes, never gave so firm a jelly that the vessel containing it could be inverted without losing the blood. Quite frequently the clotting took place in more or less isolated clumps or shreds, which after a time sank to the bottom. In other cases a continuous mass of fibrin formed in the blue liquid, sometimes floating freely, and at other times sinking to the bottom, so as to form a firm, gelatinous layer along the bottom of the glass vessel. The blood never jellied firmly throughout its entire mass, the fibrin formed floating more or less freely in the blue serum. In many cases the serum could be decanted from the gelatinous lumps of fibrin a few minutes after coagulation had begun. The coagulation takes place in a remarkably short time, and efforts made to prevent it by the ordinary methods of cold and mixture of the blood with neutral salts were unsuccessful. Blood allowed to flow directly into a cylinder packed in ice clotted almost as quickly as at the ordinary temperature. Halliburton states that the coagulation is prevented by the admixture of a large amount of saturated magnesium-sulphate solution. I tried the same experiment several times, but in each case the blood clotted, partially or completely; the cause of my failure may have been that the proportion of saturated magnesium-sulphate solution to the quantity of blood used was not sufficiently large. The serum obtained after removing the coagulum was usually of a deep blue or greenish-blue color by transmitted light, while by reflected light it gave an opaque whitish-blue color. Its reaction to litmus paper was very strongly alkaline.

Albumens of the Serum.

Numerous specimens of the serum were neutralized or made feebly acid with one per cent. acetic acid, and then slowly and carefully heated in a test tube in which a thermometer was fixed; the whole being immersed in a double water-bath made of two beakers. The neutralization of the serum gave always a slight albuminous precipitate, which was filtered off before the heating experiment was begun; the nature of this precipitate will be spoken of farther on.

The general result of the heat experiments was that the serum gave four coagulations at different temperatures. If each coagulation indicates the existence of a distinct albumen, then we have in the Limulus serum four different albumens coagulating respectively at 58°–60°C., 68°–70°C., 74°–75°C. and 78°–80°C. The coagulations at 60°C. and 75°C. were very slight, so that the albumens coagulating at 68°C. and 80°C. constitute the main bulk of the albumens of this serum, and of these two the albumen coagulating at 80°C. is the more abundant.

The first three of these albumens were completely precipitated after heating for five or ten minutes at the proper temperature; when the precipitate was filtered off, and the filtrate again heated to the same temperature, no further precipitation occurred. Quite a different result was obtained from the last albumen. When the serum was heated to 78°–80°C. for ten minutes, or even half an hour, and the strong precipitate that was formed was filtered off, the clear filtrate, when again heated to the same temperature, gave an exactly similar precipitate. In order to remove the albumen completely, this process had to be repeated from ten to twenty times. In the first experiments it was noticed that the temperature of heat coagulation during these repeated heatings rose to 84°–85°C., and it was at first supposed that this was caused by the presence of several different albumens. It was soon found, however, that this was owing to the increased alkalinity of the serum: the separation of each precipitate left the serum slightly more alkaline. If care was taken to keep the serum always just neutral or very feebly acid, the temperature of coagulation remained constantly at 80°–81°C. until all of the albumen was completely removed, and a non-

albuminous liquid remained. Since in a completely neutral serum all of these precipitates occur at the same temperature, at least within one or two degrees, the simplest supposition is that we have here only one albumen, and that the separation of a portion of this albumen from its solution in some way hinders the coagulation of the remainder. No attempt was made to precipitate the whole of this albumen by a single prolonged heating to 80°C. I am inclined to think, however, that this would not have been successful—that is, if the temperature was kept constantly at 80°C. If the temperature was raised to 85°–90°C., all of the albumen was quickly precipitated. Toward the end of these partial precipitations each precipitate became very small, appearing first as an opalescence that soon became flocculent and settled more or less to the bottom. If kept now at 80°C. for ten or fifteen minutes longer, there was no sign at all of any increase in the precipitate; while if the precipitate was filtered off, and the clear filtrate again heated to the proper temperature, a new opalescence quickly appeared which soon separated out from the liquid as a flocculent precipitate.

Halliburton states that haemocyanin of Limulus blood has the "same heat-coagulation temperature as in crustacea"—that is, 65°–66° C. This is certainly an error: Limulus blood contains one albumen coagulating at 68° C., but it contains three others besides, and the name of haemocyanin is more justly applied to the albumen coagulating at 80° C., as will be shown later.

Action of Neutral Salts on the Serum.

Ammonium sulphate added to the serum to saturation completely precipitates all of the albumens, leaving a colorless liquid, which gives no precipitate on boiling and no proteid reaction with nitric acid and ammonia. Sodium sulphate has a similar action: it precipitates all of the albumens, though it acts less rapidly than the ammonium sulphate. Magnesium sulphate added to saturation gives a very large precipitate. It was found impossible to separate this precipitate thoroughly by filtering; although the solution was repeatedly filtered through the same paper, the filtrate still came through very turbid, and gave a strong precipitate when boiled. I have

several times thoroughly saturated the serum with $MgSO_4$, putting in a large excess of the finely powdered salt, and left the solution for several days, frequently stirring it, but always with the same result: a very large precipitate was produced, and this, when filtered off, gave a turbid solution still containing albumen in solution.

The precipitate caused by the $MgSO_4$ was filtered off as completely as possible, and, after purification by re-precipitation or washing, was dissolved in water and heated in the way described for the serum. The results were somewhat contradictory, owing, doubtless, to the fact that the solutions contained different amounts of $MgSO_4$. Evidence was given of the presence of the three albumens of the serum coagulating at 68°, 75°, and 80° C. The albumen coagulating at 58°–60° C. did not appear in this precipitate, but this might have been caused by the small quantity of it which exists in the serum, and the incompleteness with which the $MgSO_4$ precipitate was filtered off. I believe that the $MgSO_4$ added to saturation precipitates all of the albumens of the serum completely, except the one whose temperature of heat coagulation is at 80° C. It precipitates this last albumen only in part. When the first three albumens were completely removed from neutralized serum by heating for some time at 75°, and the clear blue serum thus obtained was thoroughly saturated with magnesium sulphate, this last albumen was only partially precipitated. Halliburton states that all of the proteids are precipitated by saturation with magnesium sulphate, but gives no particulars.

Sodium chloride added to the serum to saturation gives only a small precipitate. This precipitate was collected on a filter, thoroughly washed, dissolved in water, and then slowly heated. Two coagulations occurred, one at 60°–62° C., very small, and one at 75° C. So that the first and third of the serum albumens are precipitated by saturation with sodium chloride.

Other Reactions of the Serum.

Portions of the serum diluted about ten times with cold water and submitted to the action of CO_2, while kept at a low temperature, gave always a flocculent precipitate. This precipitate

was filtered off, washed with water saturated with CO_2, dissolved in a 2 per cent. NaCl solution, and heated. The solution became opalescent below 70° C., but no distinct precipitate was noticed until the temperature reached 78° C. The albumen coagulating at 78°–80° C., therefore, appeared to be the only one precipitated by CO_2. The precipitate in this case, however, was found to be caused by the dilution rather than by the CO_2.

Serum diluted ten times with water, and allowed to stand, soon deposits a flocculent precipitate which, when washed and dissolved, gives, on heating, two coagulations, one at 70°–72° C., and one, much larger, at 80° C. Passing CO_2 through the diluted serum appears to diminish rather than to increase the precipitate formed. The albumen coagulating at 70°, which is only slightly precipitated by the dilution, appears to be partially dissolved by the subsequent action of the CO_2, so that on heating it gives only an opalescence. After filtering off the precipitate caused by dilution, the passage of CO_2 has no further effect. CO_2 passed through the undiluted serum gives a slight precipitate, which was not examined for its temperature of heat coagulation.

When serum is exposed to the air for some time, a precipitate forms. In several cases the serum was allowed to stand in a cool place for twenty-four hours, and the precipitate formed, after washing, etc., was heated. It gave two coagulations, one very small, at 75° C., and one at 80° C., much larger, and evidently constituting the bulk of the precipitate.

The action of dialysis on the undiluted serum was also tried. It was not possible to obtain distilled water, and rain water drained from the roof of the house into a cistern was used instead. This cistern water was only approximately free from salts, and the results of the dialysis are therefore incomplete. After dialyzing the serum in parchment tubes for thirty-six hours, with frequent renewal of the outside water, quite a considerable precipitate was obtained. This precipitate, after treatment in the usual way, was dissolved in a two per cent. NaCl solution and heated. It gave two coagulations, one at 70° C., and one at 80° C.

Neutralization of the serum with 1 per cent. acetic acid, which was always done before a heating experiment was tried,

caused a small precipitate to form. This precipitate, dissolved in a two per cent. NaCl solution, coagulated at 78°–80° C., so that a portion of this albumen is precipitated by simple neutralization or very feeble acidity of the serum. If the serum was made distinctly acid with 1 per cent. acetic acid, a strong precipitate was formed. The filtrate from this precipitate was clear at first, but soon became opalescent, and after the addition of a little more acetic acid deposited a new precipitate. The filtrate from this, also clear at first, soon gave a third precipitate. After this last precipitate had been filtered off, the solution remained clear; a portion of it, boiled, gave a small precipitate, and a portion which was neutralized gave a larger precipitate. 1 per cent. acetic acid, therefore, added to serum until decided acid reaction is obtained, precipitates nearly the whole of the albumens, and converts a portion of the remainder to acid albumen. The precipitate produced by the acetic acid is insoluble in water and dilute saline solutions.

From a consideration of the reactions given, we must conclude that the albumens of Limulus serum belong to the globulin group. They bear a closer resemblance to paraglobulin, perhaps, than to any other albumen, though they are certainly not identical with it. The partial precipitation of some of these albumens by sodium chloride, the much larger precipitate caused by magnesium sulphate, the partial precipitation by dilution with water, by dialysis, and by simple exposure to the air, are all reactions that are characteristic of the globulins.

Haemocyanin.

The blue color which this blood has in common with that of numerous crustaceans, gastropods, cephalopods, and also the scorpion, according to Lankester, has been explained by Fredericq,[4] in his paper on the Octopus, as a compound of copper and proteid, called by him haemocyanin, and similar in a general way to the compound of iron and proteid which we have in haemoglobin.

The properties of haemocyanin are not so sharply defined as those of haemoglobin. It gives no absorption bands with the spectroscope, although it cuts off a large portion of the spectrum

at either end, especially at the blue end. No one has been able to get the haemocyanin in the form of crystals. Numerous attempts made by me to obtain the coloring matter in a crystalline form were unsuccessful. The method used was to add as much alcohol as the serum would stand without showing a precipitate, and then to keep the solution at 0°C. for some days. No sign of a crystalline precipitate was ever obtained.

The chief characteristic of haemocyanin is the loss of color it undergoes when oxygen is entirely excluded. Fredericq states that in the Octopus a difference of color can be seen between the blood flowing to and from the branchiae, and when the animal is asphyxiated the blood flowing from the branchiae becomes pale. This respiratory function of the haemocyanin is not so clearly marked in the Limulus; blood taken directly from the heart is not at first blue, and when observed through the thin walls of the arteries springing from the heart, showed no tinge of blue. When exposed to the air, however, it rapidly becomes a deep blue, and if some of the blue serum is sealed in a glass tube it becomes entirely colorless after twenty-four hours. It is worthy of note, however, that when a stream of CO_2 is passed through the blue serum for quite a long time, the serum suffers no perceptible change of color, differing in this respect from the haemocyanin of some of the crustacea. The same observation was made by Gotch and Laws.* In *Callinectes hastatus* a stream of CO_2 quickly makes the blue color disappear, and shaking with air readily brings it back.

The respiratory valve of the haemocyanin in the blood of Limulus is not so evident as in the Octopus; when once oxidized it appears to be reduced with some difficulty, and normally the arterial blood is wanting in color. The respiratory function of this compound in Limulus can only be given as an inference from its behavior in other invertebrate animals, though it can scarcely be doubted that it has an active respiratory function. The want of color in the arterial blood may possibly have been caused by the fact that the animal in all cases had been for some time out of the water, and was, perhaps, partially asphyxiated.

That the haemocyanin is a compound of copper with an albuminous substance, is easily proved. When ammonium sulphate is added to saturation to the blue serum, all of the

albumen is precipitated, leaving behind a colorless liquid which gives no reaction for copper; whereas the precipitate, after being purified by reprecipitation and washing, gives a solution of a blue color similar to that of the original serum, and this, when evaporated to dryness and incinerated, gives the reactions for copper. Whether the copper is combined with all of the albumens present, or some particular one, I am not able to say. It is certainly combined with the albumen coagulating at 80°, and perhaps only with this. After heating the serum to 75°C., and precipitating everything but this last albumen, the color of the serum appears to have been but slightly, if at all, affected. It begins to disappear, however, with each successive partial precipitation of this last albumen. So that we can look upon this albumen as haemocyanin; it constitutes the largest part of the albumens of the serum, and, as stated above, gives reactions which place it among the group of globulins—a fact shown by Halliburton, and also by myself, to be true of the haemocyanin of decapod crustacea. It differs in this respect from the haemocyanin of Octopus blood, if Fredericq's observations are correct, since in this animal saturation with sodium chloride, magnesium or sodium sulphate caused no precipitation.

Coagulation.

The coagulation of the blood among invertebrates has generally been explained as the result of the coalescence of the corpuscles. Fredericq,[4] Geddes,[5] Pouchet[6] and others, hold to this view, while Halliburton in his recent paper contends that in crustacean blood, at least, coagulation is caused by the action of a ferment derived from the blood corpuscles upon a fibrinogenous substance present in the plasma before coagulation, thus making the process identical with the coagulation of vertebrate blood, according to the most generally received theory. My investigations upon the blood of Limulus have convinced me that in this animal coagulation is the result of the union of the corpuscles. As before stated, the blood when shed never set into a firm mass; the coagulum that formed was sometimes in detached clumps or shreds, and sometimes in a more or less continuous mass that frequently settled to the bottom; but in no case, when the blood

was collected in large quantities, did it jelly throughout the whole mass.

If a bit of the coagulum that formed was taken out a few minutes afterward and examined under the microscope, it was found to be composed of a mass of corpuscles, with perhaps no intermediate substance at all. Sometimes the corpuscles were irregularly packed together; sometimes, especially when a small thread of fibrin was removed which had joined two larger clumps, the corpuscles were all elongated to a spindle shape and packed closely side by side, with their nuclei still plainly visible.

I have frequently watched a drop of blood coagulate under the microscope. Almost immediately upon drawing the blood, and before one can well get a look at it through the microscope, the ovoid corpuscles become spherical and begin to send out numerous processes, which during the first few seconds, at least, appear to be slightly amoeboid. These processes unite with similar processes from other corpuscles, or with the body of another corpuscle, so that within a few minutes clumps of corpuscles can be seen all over the field, bound together by these processes, which are comparatively bold and easily seen with the microscope without the aid of staining. By the shortening of the processes the corpuscles are drawn closer and closer together, until in many cases several fuse into a common mass in which the nuclei of the individual corpuscles can still be seen, and this mass is usually connected with other corpuscles by threadlike processes. Owing to the very small layer of liquid between the cover-slip and the slide, and the fact that the corpuscles adhere either to the slip or the slide, this process is not carried so far as it is when the blood is collected in large vessels. But the explanation of the phenomenon of coagulation is furnished by the study of these microscopic preparations. The series of events, I take it, is as follows: *The corpuscles, after the blood has escaped from the vessels, almost immediately send out processes which unite with other corpuscles, or the processes given off by them, and then, by the shortening of these filaments, the corpuscles are brought together into a more or less compact mass.*

Certainly there can be no doubt that these phenomena occur in the order stated, and the only question that remains is whether or not this constitutes the whole of the process of coagulation.

I am inclined to think that it does for the reasons given above: first, that the masses of fibrin formed during coagulation, when examined under the microscope, are seen to be composed of aggregations of corpuscles, frequently packed closely side by side, with no intermediate fibrillar or gelatinous substance; second, that the coagulation of the blood when drawn in quantities does not consist in a firm gelatinization of the whole mass, as we should suppose would be the case if a fibrinogenous substance existed in solution in the plasma, and became converted into fibrin by the action of a ferment; third, the study of the coagulation of a drop of blood under the microscope shows that the corpuscles send out processes which bind the corpuscles together into masses, or form a meshwork of fibrils uniting corpuscles or clumps of corpuscles to one another. Very beautiful preparations of the coagulation can be obtained by staining the drop, after rinsing in water, with a solution of Kleinenberg's haematoxylin, washing and mounting in glycerine. Plate XVIII, Figs. 1, 2, 3, 4, 5, gives examples of the results obtained. For an explanation of the figures, I refer to the description of the plate given at the end of the paper.

The Fibrin Formed in Coagulation.

The fibrin formed in coagulation, as stated above, is composed of masses of corpuscles, the individual corpuscles at first being easily distinguished. Clumps and strings of this corpuscular fibrin were taken out, thoroughly washed, and examined as to their chemical properties. It is well known that the term fibrin has at present no very definite significance, except as the name of the substance formed in coagulation. Specimens of fibrin from the same mammalian blood under slightly different conditions may show quite different properties. The properties of what may be called typical fibrin are differently stated by the various writers. Hoppe-Seyler, in his *Physiologische Chemie*, s. 417, states that fibrin is insoluble in solutions of the neutral salts, difficultly soluble in the dilute acids, although greatly swollen by them, and slowly dissolved by the caustic alkalies; while Gamgee, on the other hand, *Physiological Chemistry*, Vol. I, p. 36, asserts that freshly prepared fibrin is soluble in 6 per cent.

solutions of potassium nitrate, and 10 per cent. solutions of magnesium sulphate or sodium chloride.

The properties of Limulus fibrin agree very closely with those given by Hoppe-Seyler for typical mammalian fibrin.

Treated with a 10 per cent. solution of magnesium sulphate for 24 hours, during 8 hours of which the temperature was kept between 35° and 40° C., the fibrin was apparently not dissolved at all. When a portion of the solution was boiled it gave only a minute opalescence, which may have been caused by a small quantity of the serum that had not been washed out. This fibrin, therefore, is almost, if not entirely, insoluble in a 10 per cent. solution of $MgSO_4$. Solutions of sodium chloride, 10 per cent., gave exactly the same result. Solutions of caustic soda, 1 per cent., in the course of a few hours completely dissolved the fibrin, with the exception of a small residue that was permanently insoluble, and consisted of disintegrated shreds. A portion of this solution neutralized with 1 per cent. acetic acid gave a large precipitate, soluble with some difficulty in an excess of the 1 per cent. acetic acid, but quickly soluble in 1 per cent. hydrochloric acid.

Bits of the fibrin left for 24 hours in a solution of 1 per cent. hydrochloric acid, part of the time at a temperature of 35° to 40° C., were much swollen and translucent, but scarcely, if at all, dissolved. A portion of the solution neutralized with caustic soda gave only a slight turbidity. *In its most important properties of solubility, then, the fibrin of Limulus closely resembles that of ordinary mammalian blood, although it is composed directly of corpuscles.* The substance of the corpuscle doubtless undergoes a post-mortem change of some sort, thereby acquiring its insoluble nature, but there is no necessity for supposing the action of a specific ferment in bringing about this change.

II.—CALLINECTES HASTATUS.

The blood of our common edible crab, *Callinectes hastatus*, was submitted to a series of experiments similar to those given for the blood of Limulus. The blood was usually obtained by cutting open the carapace and then puncturing the heart. It coagulates very quickly, though somewhat more slowly than the

blood of Limulus. In several cases the blood was allowed to drop directly into a vessel surrounded by ice, but this did not prevent coagulation, although it perceptibly increased the time of coagulation. The action of neutral salts, especially magnesium sulphate, was tried, but with very little success: the clotting took place in spite of the presence of a large quantity of saturated magnesium-sulphate solution. Halliburton, however, has succeeded in preventing the coagulation by this means. He finds that it is necessary to add to the blood at least four times its quantity of magnesium-sulphate solution. The few experiments of this kind that I attempted gave me directly opposite results, though it is possible that the magnesium-sulphate solution was not used in sufficiently large quantities. The clot from the crab's blood is quite different from that of Limulus. The blood jellies throughout its whole mass, forming a firm coagulum, so that the vessel containing it can be inverted without any danger of losing the blood. The clot when loosened from the sides of the vessel contracts, forcing out a clear, greenish-blue serum which has a very strong alkaline reaction. No second coagulation ever occurred in this serum.

Albumens of the Serum.

1. Temperature of heat-coagulation.

Portions of the serum made very feebly acid by the addition of 1 per cent. acetic acid were heated in the way described for Limulus serum. The serum showed in all cases the presence of two albumens. The coagulating temperature of one was below 60° C. If the serum was only neutral or very feebly alkaline, this albumen did not separate out, but gave only a very strong opalescence, the precipitate forming at about 65° C. When the serum was distinctly but very feebly acid this albumen coagulated at 55° C., giving a flocculent precipitate, sometimes quite large, sometimes very small. Compared with the second albumen, this albumen exists in very small quantities, and varies apparently in different individuals. After filtering off this first precipitate, the serum, when again heated, gave a second coagulation at 68° C. It is very troublesome to precipitate this second albumen. Like the albumen of the Limulus serum coagu-

lating at 80° C., it requires twenty or more separate heatings before it is entirely removed. In one case the serum was kept at 68°–78° for half an hour at each precipitation, but it was still found necessary to repeat the process some fifteen or twenty times. Towards the end each precipitate was quite small, and formed within five minutes after the serum had reached the proper temperature, and keeping it at this temperature for twenty-five or thirty minutes longer did not appear to increase it; whereas, if the precipitate was filtered off, and the serum again heated to 70° C., a new precipitate rapidly formed. Provided the serum was kept neutral, no increase in the temperature necessary for coagulation took place, the necessary temperature varying from 68° C. to 70° C. This latter albumen is the one that colors the serum blue, the color gradually disappearing as it is more and more completely precipitated. It may therefore be spoken of as haemocyanin.

Krukenberg[7] has observed the presence of two albumens in the blood of several crustacea: *Eriphia spinifrons*, *Homarus vulgaris*, *Astacus fluviatilis* and *Maja squinado*. He gives the temperature of coagulation at 64°–65° C. and 69°–71° C. It is probable that the higher temperatures he found necessary for coagulation were caused by the serum not being completely neutralized. Halliburton found, on the contrary, that the serum of *Homarus vulgaris*, *Carcinus maenas*, *Astacus fluviatilis* and *Nephrops norwegicus*, only contains one albumen, haemocyanin, coagulating at 68° C. There can certainly be no doubt that in the Callinectes two albumens are present, one coagulating at 55° C., the other at 68° C.

Action of Neutral Salts on the Serum.

Both albumens are completely precipitated from the serum by saturation with ammonium sulphate or magnesium sulphate. In order to get a complete precipitation by the latter salt, it was necessary to remove the precipitate at first formed. The serum was thoroughly saturated with magnesium sulphate, a large excess of the powdered salt being added, and allowed to stand for twenty-four hours. When filtered from the heavy precipitate that had formed, the serum came through perfectly clear and slightly blue, but after standing a few minutes it became turbid,

and deposited a new precipitate; more magnesium sulphate was added, and the liquid allowed to stand for another twenty-four hours, and this was repeated a third time before all of the albumen was completely removed. Magnesium sulphate, therefore, added to saturation precipitates at once a large portion of the albumens, but for the entire removal of the albumens it requires a long time, and acts best when the precipitate is filtered off from time to time.

Serum saturated with sodium chloride gives only a small precipitate. This, after being washed, was dissolved in a 2 per cent. sodium-chloride solution, neutralized and heated. The solution became opalescent at 60° C., but did not give a precipitate until 70° C.

Other Reactions of the Serum.

The ordinary strongly alkaline serum of the crab gave no precipitate when diluted ten times with water, but if first neutralized and then diluted, it gave a marked precipitate. If the alkaline serum is diluted, and CO_2 gas passed through it, a precipitate is also formed. When CO_2 is passed through the undiluted serum the blue color quickly disappears, and reappears promptly if the serum is shaken up with air, differing in this respect from the blood of Limulus. Specimens of the blue serum sealed in glass tubes lose their color within three or four hours, while the blood of Limulus, under the same conditions, retains its color for a much longer period. The haemocyanin of Limulus, therefore, is not identical with that of the crab; it has a higher temperature of heat-coagulation, 80° C., as compared with 68° C. for the crab, and it forms with oxygen a compound that is much more stable. The haemocyanin of the crab appears to be a decidedly more efficient respiratory medium than the haemocyanin of the Limulus, as one might expect from the more active life led by the crab.

Krukenberg[8] states that, in the crustacea investigated by him, no precipitation of albumen was caused by the addition of distilled water, by the passage of CO_2, or by saturating the serum with the NaCl or $MgSO_4$.

The experiments of Halliburton and myself show that this statement is not applicable to all the crustacea. The albumens of Callinectes, in accordance with the reactions given above, must

be classed among the globulins. There seems to have been some error in Krukenberg's observations, inasmuch as Halliburton, working on the same species, obtained a complete precipitation of the albumens by $MgSO^4$, while Krukenberg got no precipitate at all. In Callinectes $MgSO_4$ precipitates a large portion of the albumens even before the serum is completely saturated, although the complete removal of the albumens by this means is somewhat difficult.

Coagulation of the Blood.

The coagulation of the blood in the crab is not so simple a process as in Limulus. If a portion of the clot freshly formed is examined under the microscope, it is found to be composed of numerous corpuscles imbedded in a gelatinous mass, and these corpuscles are apparently not connected with each other by processes. If a drop of fresh blood is received upon a slide, and examined under the microscope, two kinds of corpuscles can be distinguished—one with large and conspicuous granules, and the other usually smaller, almost hyaline, with small and inconspicuous granules (Plate XVIII, Fig. 6). In a very short time these corpuscles lose their spherical or ovoid form, and begin to send out processes which, at first slightly amoeboid, soon unite with other processes or the body of a cell. Both kinds of corpuscles undergo these changes. Many of the corpuscles become flattened and form around themselves an envelope of perfectly hyaline substance. This is very noticeable in the coarsely granular corpuscles, in some of which the large granules entirely disappear during the process, apparently becoming absorbed. The union of the corpuscles into masses connected with one another by intermediate filaments, so conspicuous in the coagulation of Limulus blood, is much less marked here. Specimens of the crab's blood allowed to clot in thicker layers show almost no indication of these cell processes, but if stained with haematoxylin, extremely long and delicate processes can very often be traced from one cell to neighboring cells. Examples of this are shown in Figs. 7 and 8.

The intermediate substance also is apparently made up of a finely fibrillar material, and this, taken together with the processes united to the cells, and which can be seen forming when

a drop of the blood is allowed to clot under the microscope, convinced me that in the crab the process of coagulation is similar to that in the Limulus, with the exception that in the crab the processes sent out by the cells are longer and more delicate, and form a finer meshwork of fibrils, to which the firmer clot of the crab's blood is due. Halliburton, however, states that he has succeeded in preventing the clotting of the blood in the crustacea with which he worked by the use of a solution of saturated magnesium sulphate, and that after filtering off the corpuscles the remaining plasma clotted when sufficiently diluted and mixed with ferment. If the observations of Halliburton are corroborated, they will demonstrate, of course, the presence of a precursor of fibrin held in solution in the plasma.

The fibrin of crab's blood has quite different properties from that of Limulus. Caustic soda, one per cent., readily dissolves it. In hydrochloric acid, one per cent., the fibrin swells up, but is very slightly dissolved. In solutions of sodium chloride from two to ten per cent. the fibrin dissolves to a large extent, and the solution when heated gives a precipitate at 70°–71°C. The much greater solubility of the crab's fibrin in neutral solution of salts, compared with the fibrin of Limulus, is very marked.

A comparison of the blood of the crab and Limulus shows that they differ chiefly in the number of albumens contained and the properties of the fibrin formed in coagulation, and perhaps in the method of coagulation. The difference seems to me to be too wide to permit us to suppose any close relationship between the two forms, especially as they have the same general environments; but until a similar series of observations is made on the scorpion or some arachnid, we will not have sufficient evidence to make any just inferences with regard to the relationship of these forms — that is, from the standpoint here assumed.

III.—THE BLOOD OF A SPECIES OF HOLOTHURIAN.

This holothurian is very abundant at Beaufort, N. C., and its blood was investigated because it was found to contain large red corpuscles with haemoglobin in them. A description of the properties of this haemoglobin is given in an additional communication.

The liquid of the perivisceral cavity and water-vascular system of this holothurian contains two kinds of corpuscles. One corpuscle is oval, bi-convex, with a conspicuous nucleus, and of a pale straw-yellow color, from the haemoglobin which it contains. Besides the nucleus one can often see in these corpuscles one or perhaps two little masses, usually of a redder tinge than the rest of the corpuscle; they seem to be granules of haemoglobin. In addition to these red corpuscles there are a number of smaller, spherical, white corpuscles, which are granular, nucleated, and when examined under the microscope show quite active amoeboid movements. Besides these two forms of corpuscles the liquid usually contains a few quite small, oval, colorless bodies, apparently not nucleated, and now and then one sees a mulberry-like mass composed of small bodies of a red color. The meaning of these forms I do not know. Drawings of these different corpuscles are given in Fig. 9.

When the perivisceral or water-vascular liquid is received into a watch-glass and allowed to stand for a few minutes, all of the corpuscles sink to the bottom, forming a membranous-like sediment that has the appearance of an incipient coagulation. The supernatant liquid contains no albumens at all in solution. The only albumens present are those contained in the formed elements. The haemoglobin is easily dissolved out by the addition of water, but the substance of the white corpuscles exists in too small a quantity for investigation.

A drop of the liquid from one of the ampullae when watched under the microscope, showed that the incipient coagulation spoken of above is caused by the fusion of the white corpuscles. These corpuscles send out thick processes or pseudopodia which may be withdrawn, but which finally fuse with processes from other corpuscles or with the corpuscles themselves, forming what Geddes calls a plasmodium. Examples of this are given in Fig. 10. When the sediment that settles to the bottom of the watch-glass is examined, masses of fused white corpuscles are frequently found, surrounded by red corpuscles (Fig. 11). The red corpuscles exhibit no tendency to fuse, but may become entrapped in the masses of fused white corpuscles.

While in the crab's blood the union of the corpuscles is effected by processes much more delicate than in the Limulus, we have

in the blood of this holothurian apparently the most primitive method of fusion, by direct union of the cells or of thick processes given off by the cells.

In a preliminary communication of this work in the Johns Hopkins University Circular, October, 1885, the name of this holothurian was given as *Cucumaria sp.* It has since been identified by Prof. Rathbun, of the Smithsonian, as *Thyonella gemmata*, (*Pourtales*) Verrill.

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DESCRIPTION OF FIGURES, PLATE XVIII.

Figure 1.—Corpuscles of blood of Limulus Polyphemus preserved by very dilute solutions of mercuric chloride. Camera-lucida drawing.

Figure 2.—Showing the change of form of the corpuscle that takes place as soon as the blood is shed. *a*, the normal shape of the corpuscle; *b* and *c*, successive changes of *a* taking place within a few seconds. Camera-lucida drawing.

Figure 3.—From a filament of fibrin formed in the coagulation of Limulus blood. Quite frequently cords or shreds of fibrin, with the

corpuscles elongated in this way and packed closely side by side, could be taken from a vessel in which the blood had clotted.

FIGURE 4.—From a drop of Limulus blood allowed to clot on a slide and afterwards stained with haematoxylin. The whole of the preparation consists of similar groups of corpuscles united by processes. Camera-lucida drawing.

FIGURE 5.—A free-hand drawing of a clump of Limulus corpuscles the formation of which had been watched under the microscope; not stained.

FIGURE 6.—Corpuscles of *Callinectes hastatus*. *a*, the larger, coarsely granular corpuscle; *b*, the smaller, more hyaline corpuscle; *c*, two of the larger corpuscles, showing how the corpuscle becomes flattened, sends out processes and forms around itself a hyaline envelope, while the large granules disappear.

FIGURES 7 and 8.—Drawings of isolated corpuscles of crab's blood from a drop allowed to clot on a slide in a thin layer, and afterwards stained with haematoxylin. Showing the very long and delicate processes which in favorable spots can be seen to radiate from the corpuscles and unite with other corpuscles. The body of the corpuscles and part of the processes were drawn with a camera lucida; the whole extent of the processes, however, could not be drawn in this way.

FIGURE 9.—Corpuscles from the blood of the holothurian. *a*, the red corpuscles containing haemoglobin—in some of them, besides the nucleus, little masses, apparently clumps of haemoglobin, are seen; *b*, the white corpuscles of the blood; *c*, a mulberry-like mass of small, pale-red bodies whose significance is not known; *d*, two of the pale, non-nucleated, lenticular bodies frequently seen in the blood. Camera-lucida drawing.

FIGURE 10.—The fusion of the white corpuscles of the holothurian blood when allowed to clot on the slide. From a haematoxylin staining. Camera-lucida drawing.

FIGURE 11.—Mass of fused white corpuscles of holothurian blood when allowed to undergo its imperfect coagulation in thicker layers of the blood. Around the periphery of the mass are a number of red corpuscles. From a camera-lucida drawing.

NOTE ON THE PRESENCE OF HAEMOGLOBIN IN THE ECHINODERMS. By W. H. HOWELL, Ph. D.

Haemoglobin has been found in a large number of invertebrates, comprising representatives of the insecta, crustacea, mollusca, annelids (in which it is quite common), gephyrea, nemertina and hirudinea. It occurs usually in solution in the plasma of the blood, though in some few cases it is contained in the corpuscles. No satisfactory example of its presence among the echinoderms has been discovered. Foettinger, *Archives de Biologie*, Vol. I, 1880, p. 405, described, in one of the ophiurians, *Ophiactes virens*, certain red corpuscles contained in the water-vascular system which, upon examination with the microspectroscope, gave two absorption bands similar to those of oxyhaemoglobin; from this he concluded that the coloring matter of these corpuscles is haemoglobin. But other pigments—turacin, for instance—are known to give very similar, if not identical, bands, so that this single observation, as Krukenberg remarks, cannot be considered as demonstrating the presence of haemoglobin.

During the summer's work at the Marine Laboratory, the writer's attention was directed by Mr. H. F. Nachtrieb to a holothurian *Thyonella gemmata*, whose perivisceral liquid is in some cases of a bright red color. Examination of this liquid, and of the contents of the water-vascular system, showed that the coloring matter has most of the properties of haemoglobin, though certain differences in its chemical reactions have led me to believe that it is not identical with the haemoglobin of vertebrate blood.

The water-vascular system and the body-cavity contain, besides colorless amoeboid corpuscles, a large number of oval, nucleated, bi-convex blood discs of a pale red color. When a specimen of the water-vascular liquid is caught in a watch crystal, these corpuscles soon settle to the bottom, forming a

membranous-like sediment. If the supernatant liquid is decanted, and the sediment treated with water and then filtered, a beautiful blood-red solution is obtained. This solution, when examined with the spectroscope, gives the two oxy-haemoglobin bands; the wave length of the middle of each band was determined, and was found to be identical with that of the corresponding band of a solution of oxy-haemoglobin of human blood of the same strength. Addition of Stokes's reducing solution causes the two bands to disappear, and brings out the single band of reduced haemoglobin. By shaking the solution with air, the two oxy-haemoglobin bands can again be obtained. The corpuscles, when treated with glacial acetic acid and salt, give well-marked haemin crystals, though these crystals do not form so readily as in vertebrate blood. When incinerated and tested with ferrocyanide of potassium, they give the reaction for iron.

These properties are quite sufficient to show that this coloring matter is a compound similar to ordinary haemoglobin, and justify its right to the title of haemoglobin. In two respects, however, it differs from the haemoglobin of vertebrate blood. An aqueous solution, when heated, coagulates at 58°–60°C., giving a heavy brown precipitate, the filtrate from which is perfectly free from albumen. Haemoglobin solution from vertebrate blood, on the other hand, coagulates between 70°–80°C. A solution of this haemoglobin is also precipitated by the addition of dilute (1 per cent.) acetic acid, which is not true of vertebrate haemoglobin. Addition of alcohol or of ether quickly precipitates and decomposes the haemoglobin.

It appears, then, that we have here a compound similar in structure and in some of its most essential properties to the haemoglobin of vertebrate blood, but differing from it slightly in the albuminous portion of the molecule. It seems quite possible that similar differences may be found when the haemoglobin of other invertebrates is more carefully examined. All efforts to obtain haemoglobin crystals were unsuccessful.

The respiratory value of this pigment cannot be doubted. It functions not only through the ambulacral feet scattered over the surface of the body, but also through the respiratory tree, the ramifications of which are bathed in the perivisceral liquid. The respirations of the animal when undisturbed are very

regular, consisting usually of three inspirations followed by a single prolonged expiration, the whole respiratory act being repeated from three to four times a minute. Efforts to increase the respiratory rhythm by heating the sea-water surrounding the animal were not successful.

In a preliminary communication of these observations in the *Johns Hopkins University Circular*, October, 1885, the name of this holothurian was given as *Cucumaria sp.* It has since been identified by Prof. Rathbun, of the Smithsonian, as *Thyonella gemmata*, (*Pourtales*) Verrill.

ON THE SOCALLED "NEW ELEMENT" OF THE BLOOD AND ITS RELATION TO COAGULATION.

By GEO. T. KEMP, A. B., Fellow in Biology, Johns Hopkins University. With Plate XIX.

Whenever a figure appears in heavy type, the number refers to the corresponding number in the Bibliography under the name of the author.

In 1878 Hayem called attention to an element of the blood, whose existence and significance seem to have been almost wholly unrecognized. Hayem called these elements *hæmatoblasts*, and endeavored to show that they were early stages in the development of the red corpuscles. He also pointed out that they probably are connected in some way with the coagulation of blood; but as the theory of their hæmatoblastic function did not meet with general acceptance, the whole work seems to have been passed over with less attention than it merited.

Much greater prominence was given to the subject, when, in 1881, Bizzozero claimed independent discovery of the same elements, and emphasized particularly their connection with coagulation. He attacked the theory then in vogue as to the relation of the leucocytes to coagulation, and called forth many severe criticisms, which in turn were answered; thus the question was brought prominently to the attention of histologists. The result was several important investigations; but the irreconcilable results obtained by different observers, rendered it desirable that the whole matter should be made the object of further research. I was therefore led to take up the study of this question, and the results of my work thus far, form the material for this paper.

Before going into a discussion of the subject, which will involve the results of other observers, I will give a brief description of the "new element" as observed by myself, in order that what is to follow may be better understood.

In describing it I shall adopt the French name *plaque*, as it is short, more or less appropriate, and not liable to be confused

with any other name which may be used in connection with this subject.

If a drop of osmic acid be placed on the finger, and the finger pricked with a needle through the drop, the elements of the blood will all be hardened and preserved in their natural appearance immediately upon leaving the vessel.

If now a thin film of the blood mixed with osmic acid be examined under the microscope with a *good lens* magnifying about 600 to 800 diameters, the plaques may be seen floating in the plasma among the red corpuscles and leucocytes.

They are very pale and homogeneous structures, varying greatly in size, but mostly about one-third or one-fourth the diameter of the red corpuscles. When seen in surface view, they may appear either circular or elliptical, and seem at first sight to be flat, but a more careful observation with a fine objective, will reveal the fact that they are biconcave, although not as much so as the red corpuscles. (See Plate, Fig. 1, and photograph.) This is more plainly marked when we examine them seen on edge, in which case they show the characteristic dumb-bell shape presented by biconcave bodies when viewed in this position.

The form of the plaques, when hardened by the above method, never undergoes change. This is not the case, however, in blood drawn and allowed to clot. To study the plaques under these circumstances, the following method may be adopted: The finger is pricked and a good-sized drop of blood squeezed out. This is taken immediately upon a cover-slip, and then as quickly as possible most of it is washed off by a jet of .75 per cent. NaCl solution from a wash-bottle. The cover-slip is now placed on a slide, and transferred to the microscope stage with as little loss of time as possible. The plaques have the property of sticking to the slip, while the other elements are easily washed away by the jet, so that upon examination the whole field will be seen to be filled with plaques, some of them isolated, but most of them grouped in masses consisting of from two or three to a dozen or more. (Plate, Figs. 2 and 3, and photograph.)

They are now not pale and homogeneous, with a symmetrical outline, but appear glistening and granular, and their contour, instead of being regularly oval or circular, has become jagged. These changes are the more marked the longer the time which has

elapsed before the preparation is observed; and they may be seen to take place step by step while a preparation is being watched. The form of the plaques continues to undergo change, until finally, where they are grouped together, only a granular mass is found, in which the individual plaques can no longer be clearly distinguished. (Plate, Figs. 4 and 5.) *Pari passu* with these changes, processes are seen which run out from the granular masses; and when coagulation sets in, these processes are nearly always found to be continuous with threads of fibrin. (Plate, Figs. 5 and 6.)

The threads of fibrin are sometimes deposited as long needle-shaped crystalloids which are often seen lying free in the field, and not in connection with the granular masses; but the greater number are formed most thickly around these masses, from which they often radiate as centres. (Plate, Figs. 5, 6 and 7.)

If too much of the blood has been washed away by the jet of salt solution, no formation of fibrin will take place; if there is any part of the field where the blood still remains thick, the best observations can be made on the edges of this area.

The preceding description, I think, is given in sufficient detail to indicate what I mean by the term *plaques*.

§ 1.

Historical.

Among the earlier observers, Müller, Mandl, Henle, Wharton Jones and others, have described various colorless elements existing in the blood; and it is not unlikely that they saw the plaques, more or less modified; but their descriptions are not sufficiently minute to enable us to make a positive decision.

Gerber[1] has described in the blood of mammals, bodies which he calls free nuclei, having a diameter of $\frac{1}{540}$ line ($=$ 4.1 μ). Arnold[2] and Andral have also been recognized as possible discoverers of the plaques.

[1] Gerber. Allgemeine Anatomie. Quoted by Zimmerman, 2, 226.

[2] Arnold (Anatomie, Band I, p. 181) has been referred to by Zimmerman (2, 226) and Hayem (8, 581) as having probably seen the plaques. Hayem gives Arnold's measurement as $\frac{1}{1500}$ foot (which I take to be a misprint) and Zimmerman gives it as $\frac{1}{1500}$ zoll ($=$ about 16.7 μ), which is entirely too large for a plaque. Zimmerman thinks that the structures described by Andral (Essai

Simon,[1] in studying blood drawn into a solution of potassium ferrocyanide, found small bodies which he took to be molecules of fibrin, but which were probably plaques, and other elements deformed by the reagents that he used.

Donné[2] (1842) described and figured the plaques so as to leave no doubt as to their identity. He calls them *globulins*, and says they belong properly to the chyle, from which they are supplied to the blood. He describes them as small white particles, or little rounded grains, isolated or irregularly agglomerated, with a diameter of not more than $\frac{1}{300}$ millimeter ($= 3.3\ \mu$), and says they are important as being the "premiers éléments des globules sanguins." He also noticed the tendency of the plaques to adhere to each other.

The *globulins* of Donné are not the same as the globulins of Milne-Edwards, nor of Robin; the former using the word to denote small, fatty particles found in the blood especially after a meal, and the latter using the word with reference to the smallest variety of leucocytes.[3]

To Zimmerman (1846) belongs the credit of first having studied the plaques with the aid of micro-chemical reagents. He (**2**, 227) repeated the experiments of Simon by drawing blood into a solution of potassium ferrocyanide, which prevents clotting, and then examining with the microscope. He found small bodies, occurring "by the billion," which he describes as quite colorless and more or less strongly refractive; they have not a well-defined contour, and when out of focus appear as dark points. They vary in size from $\frac{1}{1000}$ to $\frac{1}{400}$ of a line ($=$ about $2\ \mu$ to $5.2\ \mu$). He does not agree with Simon that they are molecules of fibrin, and calls them "Elementarkörperchen," because they cannot be

d'hématologie pathologique, p. 32), as molecules of fibrin are the same as those mentioned by Arnold, while Hayem (8, 582) thinks that Andral's molecules of fibrin are likely plaques, because of their small size. I was not able to refer to the papers of Arnold and Andral, so that I cannot reconcile the statements of Zimmerman and Hayem. Possibly the measurements referred to granular masses, which Arnold may have explained, without the explanation being quoted by Zimmerman or Hayem.

[1] Simon. Anthropochemie.

[2] Donné. Comptes Rendus de l'Académie des Sciences, 1842 (Vol. 14, pp. 366–368), and Cours de Microscopie, etc., 1844, p. 85; and Atlas, Plate 6, Fig. 21.

[3] See Hayem, 8, 583.

broken down into any other smaller-formed element, and because, as he claims, they have power to grow by intussusception and form new cells. As to their origin, he supposes that they, like the white corpuscles, belong properly to the lymph, from which they are supplied to the blood.

It is more than probable that under the name of elementary corpuscles, Zimmerman has included not only the plaques, but also other bodies, especially certain granules often found in the blood, which are partly normal and partly the result of some methods of preparation. He speaks of elementary corpuscles as occurring in the blood of a frog; and describes in addition the nucleated plaque (found by later observers in the blood of all ovipara) as an intermediate stage in the formation of red corpuscles from the elementary corpuscles. He also claims to have seen the elementary corpuscles in defibrinated blood.

These inaccuracies have led some histologists to question whether Zimmerman really found anything but detritus, resulting from his methods of preparation; but his description of the behavior of the elementary corpuscles with certain reagents—*e. g.*, water, caustic potash, salt solutions, etc.—corresponds so nearly with the reactions of the plaques under similar conditions, that we can hardly doubt that he had plaques under observation.

Beale (1864), (**1**, 42) describes as existing in the blood, "numerous corpuscles differing very much from the ordinary red corpuscles in size, color, and refractive power. They are much smaller than the latter; they exhibit a granular appearance and are colorless. They might be described as small white corpuscles, but many are much smoother than the colorless corpuscles. It is not easy to see these corpuscles unless the blood is examined by powers magnifying upwards of 1000 diameters. Such corpuscles are exceedingly faint, and can only be distinguished if great care be employed. . . . . *The small, faintly granular corpuscles are colored by carmine*,[1] while the ordinary red corpuscles . . . . are not."

At first, from the above description, it seems as if Beale had reference to the plaques; but the fact that the structures he describes stain with carmine excludes this supposition.

Beale (**2**, 48) also describes other structures which occur "*only*

[1] The italics are mine.

in the blood of man and the higher animals," and which are of the same refractive power as the white corpuscles. These particles he thinks may develop into white or red corpuscles. He further mentions (**2**, 60) small bodies, which he believes to be a kind of white corpuscle, which stick to the slide, while the red corpuscles are drawn hither and thither by the current. These descriptions may well apply to the plaques.

The "granular masses" described by Max Schultze (1865), which have been more or less well known to histologists ever since, are undoubtedly plaques, as may be seen from his description. He says (**1**, 36 and 37), "I find more or less plentiful in my blood and the blood of many others, . . . clumps of colorless balls varying in size accordingly as they are made up of few or many individuals. The latter measure .001—.002 mm., and occur also singly in the blood, but more frequently they are found sticking together to form an irregular, finely granular mass. The balls are quite colorless, homogeneous or finely granular and pale, ... and hence, as also on account of their small size, they can only be made out with a good lens.

"They are, however, not always regularly ball-shaped: they are often angular and drawn out, and then they present a sharper contour and a clearly granular appearance. . . . . It looks as though they may have come from the leucocytes, but of this we must remain uncertain as long as we are in the dark as to the fate of the latter."

He also obtained the characteristic reactions of plaques with water, acetic acid and other reagents.

Riess (1872), (**1**, 240) examined blood drawn by venesection, with as little loss of time as possible, and found "Stäbchen und helle Kügelchen von ähnlichem Glanze mit den farblosen Körperchen."

The "Kügelchen" are mostly round, but sometimes angular. They are usually about $\frac{1}{10}$ the diameter of red corpuscles (.7–1.5 μ), but they vary from very minute bodies to half the size of the red corpuscle. This last statement makes it somewhat doubtful whether Riess has made the necessary distinction between the plaques and the smaller varieties of granules found in the blood. He also claims to have found all stages between leucocytes and granular masses of plaques.

In 1873 Ranvier laid a communication (**2** and **31**) before the Société de Biologie, on the "Formation of Fibrin in Blood Removed from the Vessels." He describes the threads of fibrin as radiating from granules, or groups of granules, which he thinks are chemically identical with fibrin. From references made to these granules in later publications we know that he had reference to the plaques, which he has also seen free (**1**, 215).

In connection with Ranvier's communication, Vulpian (**1**, 94) gave the results of some observations which he made on blood. He examined the blood of many persons, and found that there were always present small corpuscles, either singly or in groups, which quickly adhered to the slide, and were not carried about by currents, like the other elements. When coagulation takes place, the threads of fibrin are often seen to radiate from prolongation of these corpuscles, or from the edge of groups into which they have become collected. He identifies these corpuscles with those described by Riess, and takes occasion to combat the statement of Riess that they are the result of pathological conditions, maintaining that he has found them in the blood of healthy persons as well as in the blood of the sick.

Bearing also on the relation of the plaques to the fibrinous network are the observations of Nedsvetzski (**1**, 147–150), made independently of the work of Ranvier and Vulpian, and published about the same time. He describes small, homogeneous bodies about the size of the granules of the leucocytes, which he regards as normal constituents of the blood, and gives them the name of "Blutkörnchen" or "Hæmococci."

After fibrin has been formed, these small bodies are found like knots in the meshes of the network. He also says that these bodies possess the power of movement.

The Proceedings of the Royal Society of London for 1874 contain "An Account of Certain Organisms Occurring in the Liquor Sanguinis," by Osler, in which he describes certain interesting changes which take place in the granular masses when heated in serum to 37° C. In the same communication Osler secures for himself the credit of having first discovered the plaques in the bloodvessels. His observations were made on connective tissue from the back of young rats, in the vessels of which the plaques were seen scattered freely in the blood among

its other elements. The figures which accompany Osler's article are very good.

From this time until the publication of Hayem's work, the only literature bearing on the subject are the papers by Schmidt (**2** and **3**), Semner (**1**) and Boettscher (**1**). Schmidt and Semner examined "the granular masses of Max Schultze," and described them as being derived from a peculiar corpuscle not at all like the plaques.

Boettscher (**1**, 298 and plate), who believes the red corpuscles to be nucleated, has figured structures found by him among the red corpuscles, which he regards as nuclei set free from the latter; but which, judging from his figures, are probably plaques.

To show what a confusion of ideas prevailed in regard to this subject before Hayem's work came out, I cannot do better than follow Hayem in quoting from Robin (*Leçons sur les humeurs normals et pathologiques*, 1874). After reviewing the work of Riess and Vulpian, he says: "Il est de fait qu'avec un peu d'attention on trouve de ces globules sur presque tous les sujets, bien qu'en très petit nombre. On en voit même circuler de loin en loin entre les hématies et les leucocytes, sur les batraciens et les poissons vivants. Ces corpuscules se trouvent soit isolés, soit réunis en petits groupes. Ils sont larges de 0.002 à 0.006, c'est-à-dire que certains atteignent presque le volume des hématies et même des leucocytes. Ils sont incolorés, hyalins, homogènes ou à peine grenus. Parmi ceux-ci il en est qui se déforme lentement comme les leucocytes. Pour ces derniers l'action de l'eau et de l'acid acétique sous un fort grossissement montre aisément qu'il ne s'agit là que des leucocytes encore petits et en voie d'evolution, qu'il s'agit en d'autres termes de ceux des leucocytes que M. Donné (1844) a décrits sous le nom de *globulins*, larges de 0.003."

With Hayem's work (1878) begins a new era in the history of the plaques, for, by the careful and thorough investigation to which he subjected them, he was not only able to remove all doubts as to their existence and identity, but he also traced their close connection with the process of coagulation, and has advanced some very suggestive speculations regarding their affinities to the red corpuscles.

Bizzozero (1881 and 1882) so confirmed and extended the

observations of Hayem by new and ingenious methods, that all the work done later, has been carried on with a more definite understanding of the subject, and the results of the observations may be considered as confirming or conflicting with those of Hayem or Bizzozero.

Before concluding the historical chapter of this article, the claim of Norris to the discovery of the plaques should be considered.

Norris bases his claim on a communication read before the Birmingham Philosophical Society, in 1879, in which he says (**2**, 163): "There exist in the blood of mammalia . . . . colorless, transparent, biconcave discs *of the same size* [1] as the red ones. Between these two kinds of biconcave discs others are demonstrable having every intermediate grade of *color*.[1] . . . They often present themselves as small spheres, at other times as discs, and at others, liquid-like, they take the shape of the interstices in which they lie."

In a later article (**3**, 562), Norris speaks of Hayem's haematoblasts as fragments of one form of his corpuscles.

From a comparison of these descriptions with those given at the beginning of this paper, I think it will appear evident that what Norris saw was not the plaques, but red corpuscles which had lost their haemoglobin.

§ 2.

Histology of the Plaques in the Blood of Mammals, with Methods of Preparation.

The plaques have been found under different circumstances by so many observers (see historical section), that it is now needless to enter into a discussion as to their presence in the blood—at least after it has been removed from the bloodvessels.

The method of preparation may be varied to advantage according to the point which we wish especially to make out. The following may be recommended as giving very satisfactory results:—

A drop of the preservative fluid is placed on the finger, and the finger pricked through the drop, so that the blood may come

[1] The italics are mine.

in contact with the reagent immediately upon leaving the vessel. The drop of blood thus mixed with the reagent is next taken on a cover-slip, and the latter laid on a slide and examined. To prevent evaporation, melted paraffin may be painted around the edges of the slip.

The part of the finger from which the blood is to be drawn should be washed with water, alcohol and ether, in the order named.

Whenever it is necessary to run any fluid under the slip by suction with filter-paper, it can most easily be accomplished by putting the minutest part of a drop of very thick balsam on each of the four corners of the cover-slip. When laid on the slide the balsam should be on the under side of slip. By this arrangement the slip is held to the slide by the balsam, and will not be floated about by an excess of the fluid run under. It also prevents the slip from lying so close to the slide as to interfere with the filter-paper readily absorbing the fluid. Other observers have used paraffin, but I find thick balsam rather superior.

On collecting blood directly from a needle-prick into a suitable liquid, the plaques are preserved in their original form, but their relative number is small, and they do not readily attract attention lying among the more prominent corpuscles. Their presence in the blood may be demonstrated more strikingly by proceeding as follows: A large drop of blood is squeezed from the finger and taken directly on a cover-slip. The surface of the cover-slip to which the blood is adhering, should next be touched three or four times to the surface of some .75 per cent. salt solution in a watch-glass, or washed with a jet of the same solution from a wash-bottle, until nearly all of the blood is apparently washed off. This part of the proceeding should be finished as quickly as possible, and the cover-slip placed in a watch-glass of osmic acid for about twenty to thirty minutes or longer. It should then be washed by leaving it in a watch-glass of water for several minutes, after which it may be mounted in acetate of potash and examined.

The plaques are seen in great numbers all over the field. Sometimes they may be found singly, but more frequently they are seen in groups, the size of which depends upon the length of time between drawing the blood, and getting it into the osmic

acid. (See photograph.) If this be very short, the masses are small, and the individual plaques of which they are composed may be plainly distinguished; but the longer the time, the larger, as a rule, will be the masses, and the less distinctly can the individual plaques be seen.

For hardening and preserving the plaques, I think 1 per cent. osmic acid is the best reagent that can be employed. Instead of osmic acid we may use Hayem's solution, the formula for which is:—

Distilled water,	200
$NaCl$	1
Na_2SO_4	5
$HgCl_2$	0.5

I prefer, when using Hayem's solution, to dilute it with $\frac{1}{10}$ its volume of 75 per cent. NaCl solution. This avoids the disadvantage of a precipitate if the fluid should evaporate or become more concentrated, and preserves the plaques very well. The plaques show very plainly when examined in Hayem's solution, their appearance being more striking than when seen in osmic acid; but we are more apt to get very slight irregularities of outline, together with minute granules deposited on the face of the plaque, which are less frequently seen in osmic acid preparations. This is probably due to Hayem's fluid acting somewhat less quickly than osmic acid, thus allowing to appear the first changes which the plaques undergo in breaking down. I have also tried Bizzozero's fluid, and find it an admirable medium for studying the changes undergone by the plaques in breaking down, as the changes go on very slowly in this solution. Bizzozero's fluid is .75 per cent. NaCl solution, to which methyl violet is added. He recommends the ratio of methyl violet to salt solution 1:5000. I did not determine the ratio of the violet to the salt solution in the fluid which I found to give the best results, but I think it contained relatively more of the staining element than that recommended by Bizzozero.

I have also tried the method of preservation by drying. Preparations were dried both by spontaneous evaporation and carefully over an alcohol flame, but the results of this method were so far inferior to those obtained by hardening in osmic acid or Hayem's fluid, that I did not employ it to any great extent. If the blood is first hardened in osmic acid and then dried, the

results are much more satisfactory, although then the drying process is unnecessary.

As mounting media, I have tried the following:—Canada balsam, Dammar varnish, glycerine, glucose, acetate of potash (saturated solution) and Hayem's fluid.

Balsam and Dammar varnish were generally used with the dry specimens. They may either be mounted directly (if perfectly dry), or passed through turpentine or xylol. The latter method is rather preferable. Balsam and Dammar were also often used with specimens stained in an alcoholic fluid, but in general it is best to avoid the use of alcohol, as it appears to cause a slight shrinkage even after hardening with osmic acid.

Glycerine is not the best medium for mounting unstained plaques, especially when hardened in osmic acid, as they lose somewhat in clearness. For stained specimens it works very well.

Glucose makes a very satisfactory temporary mounting material. It is used in concentrated aqueous solution, and has the advantage of becoming perfectly hard and requiring no cement.[1]

Acetate of potash is probably the best mounting medium of all those employed. In it both plaques and fibrin threads stand out clearly and sharply defined.

In addition to these regular mounting media, the plaques may be preserved for a time in Hayem's fluid. This does very well for some weeks, but in specimens several months old there is often a coarsely granular precipitate, apparently from the plasma, and sometimes crystals are deposited.

The description which I have given of the plaques in the introductory pages of this article, agrees for the most part with the descriptions of previous observers, but there are some points of difference to which it is desirable to call attention.

In the first place, their biconcavity is still a subject of dispute. Bizzozero (**7**, 18 and **3**, 357) asserts most positively that they are flat, but says they may become biconcave when treated with strong salt solution; while Löwit (**3**, 108) says that they are

[1] This was found to hold good for several months, but in most of the specimens examined after the lapse of a very hot summer, the glucose was found to have crystallized out, and the specimen consequently was ruined. This method cannot, therefore, be recommended for permanent preparations.

never biconcave in blood drawn into salt solutions, but that under certain conditions a biconcavity may be seen in them when examined in peptone blood. Hayem (**5**, 695 and 697) and Laker (**1**, 177), on the other hand, uphold the view that they are normally biconcave, while Schimmelbusch (**2**, 217) says they are not biconcave when examined circulating in the capillaries of the mesentery, but become so when the blood is drawn into Hayem's fluid. As I have never examined the circulation in the mesentery, I am not prepared to endorse or dispute this statement; but I can say that in addition to seeing them on edge and making out their characteristic dumb-bell shape, I have succeeded several times in observing them rolling over and over in a very slow current, so that at least in osmic acid and Hayem's solution I was able to convince myself beyond all question of their biconcavity. The biconcavity also appears in the photograph, which was made from a specimen stained with Bismarck brown.

Bizzozero also describes the plaques as granular, and not homogeneous. This is due, I think, to the method which he employed. If he had drawn the blood directly into osmic acid, he would have found the plaques homogeneous.

The sizes, as given by different observers, vary within wide limits, but the mean of each of them generally corresponds pretty well with the mean of the others.

Osler gives their measurement as one-eighth to one-half of that of the red corpuscles, which I think expresses with sufficient accuracy their extreme variations in size. Hayem (**5**, 703) at first describes the plaques as homogeneous and non-nucleated, but later (**17**, 480 and 481) he changes his opinion and states that they contain a nucleus, which may be brought into view by staining with haematoxylin. He gets his best results from drying a film of blood on the slip—a method which I have never considered as free from objection, especially when heat is applied to effect the desiccation.

In a later article (**18**, 372) he again reiterates his statement in regard to the nucleus of the plaques, and this time with more confidence than before. At the same time, he expresses himself in favor of the view that red corpuscles are also nucleated.

Afanasef investigated this question after Hayem, and comes

to the conclusion that what Hayem regards as a nucleus is really only a precipitation of granules in the centre of the plaque. Schimmelbusch (**2**, 225) takes a view very similar to that of Afanasef. I have some very satisfactory specimens of plaques stained with haematoxylin; some are mounted in acetate of potash, others in balsam, but none were preserved by the drying method. A thorough examination of these preparations has failed to reveal a nucleus, but the characteristic appearance of a concave body when seen full on the surface was presented. This was rendered more prominent than in the unstained plaque, and in so small a structure, it is possible that an appearance of this sort may have been taken for a nucleus. Preparations stained with Bismarck brown and magenta show the same thing.

The same question as to a cell-membrane may be raised with regard to the plaques, as in the case of the red corpuscles.

The plaques, under certain circumstances, will shrivel and become crenate, just as the red corpuscles; and it is quite a usual thing to see both plaques and red corpuscles caught at one end and drawn out by a current so as to present a long, pear-shaped or even threadlike appearance. (Plate XIX, Fig. 1, *d.*)

Hayem (**5**, 696 and 717) described the plaques as containing haemoglobin. In this he was supported by Mayet (**1**, 243 and 244), and by the work of Laptschinsky (**1**, 658), who describes corpuscles about one-third the size of the red corpuscles, which are sometimes more strongly colored than usual, and sometimes colorless.

Hlava also (**1**, 403) has seen circulating in the mesentery of rabbits, small, mostly round elements colored by haemoglobin, which are either young red corpuscles (which he doubts) or fragments of old ones. These are not the true plaques, for he has described the latter (**1**, 404), and says they are colorless.

Riess (**1**, 224), Bizzozero (**3**, 352), Laker (**1**, 200), Hlava (**1**, 403), Halla (**1**, 220), and Schimmelbusch (**2**, 217), all take the view that the plaques are without color.

Laker has noticed the "teinte verdâtre" of Hayem, but thinks it only a shimmer from the surface. He has seen the same thing in the leucocytes. Another proof that the color is not proper to the plaques, is the fact that when seen in groups, one lying over the other, the color is no more intense than when

seen singly or isolated. This has also been noticed by Laker (**1**, 179), and by Halla (**1**, 220), as well as by myself.

Another interesting relation between the plaques and red corpuscles has been pointed out by Hayem (**5**, 728), Bizzozero (**3**, 357), and Lavdovsky (**2**, 64). They find that in different animals the size of the plaques always varies in the same ratio as that of the red corpuscles.

To these many interesting points of relationship between the plaques and red corpuscles, I can add that, in trying to get a preparation from which to make a photograph of the plaques, I tried ten different staining fluids on the blood after hardening in osmic acid, and *in every case* the plaques and red corpuscles stained proportionately.

In addition to the points of resemblance already given, Hayem (**10**, 565) also mentions a somewhat complicated process of drying, washing and staining in which the plaques deport themselves exactly like the red corpuscles.

The many prominent marks of relationship between the plaques and red corpuscles, together with the idea that they contained haemoglobin, prompted Hayem to advance his haematoblast theory: that the plaques are early stages in development of the red corpuscles, the gap between the largest plaques and smallest red corpuscles being filled by elements to which Hayem gives the name "globules nains." In support of his haematoblast theory, Hayem (**11**, 120) has found that in pathological conditions of the system where "new blood" is demanded, we always find the plaques in increased numbers— a fact which had previously been stated by Riess (**2**, 696).

The sources of error in all numerical determinations of the plaques are very great, on account of the tendency which these elements have to adhere to each other or to any foreign body with which they come in contact. I was early led to see that for this reason numerical determinations where the blood had to be measured, were practically of little value. The same point has been made by Schimmelbusch (**2**, 226–231), who, in addition to the results of his own observation, has shown that enormous variations exist in the results of the same observer, while different observers have obtained diametrically opposite results, in determining the number of plaques in certain pathological con-

ditions. This point was also noticed by Howlett (**1**, 224). I think the only way to obtain a reliable numerical determination of the plaques would be to prick the skin through a drop of osmic acid, examine a thin layer of this mixture, and count the relative number of plaques and red corpuscles. A separate determination would then have to be made by the usual methods for the red corpuscles, from which the number of plaques could be calculated. Even this method would not be free from error, for Hayem (**26**) has shown that enough plaques adhere to the edges of the wound to make an appreciable difference in the extravasated blood.

Probably the most suggestive observations in support of the haematoblast theory were made by Hayem (**4**, 330), who has found the plaques in the vaso-formative cells of the mesentery of newly-born kittens, where the young red blood corpuscles are in process of development, while both Hayem and Pouchet (**1**, 97) have described intermediate stages between the plaques and the red corpuscles.[1]

We cannot regard the haematoblast theory as proved, but we must acknowledge that the relationships between the plaques and the red corpuscles are most striking; and it seems as though this is the most plausible explanation afforded.

§ 3.

Micro Chemistry.

In studying the micro-chemical properties of the plaques, a combination of methods was used.[2]

With water the plaques swell up into large bladder-like structures, which consist of a very pale, hyaline sphere with granules collected at one point on its surface. The hyaline part is not easily distinguished in water without a very fine lens. By running iodine under the slips, the bladders may be studied to much better advantage. If the blood be so completely washed away that no fibrin will form, the bladders will begin to disappear

[1] The observation of Hlava just mentioned may also be taken in support of Hayem and Pouchet, the colored bodies which he distinguishes from the plaques, being plaques containing hæmoglobin, and on the way to form red corpuscles.

[2] Pp. 301–303.

after a day, although at the end of three days some of them may still be found, especially the smaller ones. (Plate, Figs. 8–12.)

Acetic acid (.1 per cent.) acts like water, only more powerfully.

Concentrated acetic acid readily dissolves the red corpuscles and plaques. The white corpuscles are gradually dissolved, the nuclei resisting the action of the acid the longest.

Concentrated solution of potassium hydrate quickly dissolves everything in the field except the granules of the coarsely granular leucocytes.

Solution of magnesium sulphate (27 per cent.). If this solution be used on the finger (p. 301), the plaques will remain without breaking down for a considerable length of time.

Cold.—One of the best means of studying the breaking down of the plaques is to observe them in .75 per cent. sodium chloride solution or Bizzozero's fluid at a reduced temperature—*e. g.* 6° C. This will retard the clotting so that the different stages may be conveniently observed.

A very low temperature—*e. g.*, —1 to +2.5° C.—will prevent coagulation. If a preparation be kept at this temperature for a few hours, and then the temperature be raised, the plaques will break down, but no fibrin will be formed. This is interesting, for, as Hayem (**5**, 708) has pointed out, there must be some deep-seated chemical change produced by the cold, which prevents the plaques from yielding that which appears to make them essential to the process of coagulation.

Concentrated solution of mercuric chloride preserves the plaques, but causes a precipitate in the blood which renders its use objectionable.

Of the different staining fluids, methyl violet, gentian violet, and strong fuchsin, stain the plaques readily and deeply. They should be used in very dilute solutions, especially the methyl violet.

Iodine, when run under the slip, appears to stain the plaques, and makes them stand out very satisfactorily. It is particularly adapted for studying the bladder stage. The coloration from iodine is not, however, a true stain, for the last traces of it may be washed away by water even after the iodine has been allowed to act for thirty-six hours. Bismarck brown, magenta, Kleinen-

berg's haematoxylin and aqueous haematoxylin, give very good, and for permanence the best, staining of the plaques, but a longer time is required than for methyl violet, gentian violet or fuchsin. Eosin will also stain them, but to get satisfactory results it is necessary to use a tolerably strong solution and allow it to stand from twelve to fourteen hours. Aniline blue-black, borax carmine, Frey's carmine and picro-carmine, do not stain the plaques even in forty-eight hours.

I also used a double staining fluid of carmine and indigo-carmine, recommended by Shakespeare and Norris,[1] which is claimed to stain green any cell which contains haemoglobin. Hoping to find another method of determining whether the plaques contained haemoglobin, I tried it several times, but without success.

§ 4.

Origin of the Plaques.

The question now arises: Are the plaques normal and independent elements of the blood, or have they been derived from the other elements of the blood, either normally or by the methods of preparation?

This is a subject on which the greatest diversity of opinion exists, all of the following views having been held, and experimental evidence adduced to support them:

1. They are haematoblasts, or young red corpuscles.
2. They are derived from the red corpuscles.
3. They are derived from the white corpuscles.
4. They are nuclei floating free in the blood.
5. They are fibrin.
6. They are globulin depositions produced by cooling of the blood.
7. They are independent elements.

While the red and white corpuscles were the only recognized histological constituents of the blood, it was most natural for observers to refer the plaques and granular masses to one of these elements, without considering the possibility of the plaques being independent elements themselves.

[1] Amer. Jour. Med. Science, Jan., 1877.

The theory that they are derived from the red corpuscles has received but little support. Boettscher (**1**), who believes the red corpuscles to be nucleated, thinks that the plaques are free nuclei from them. Ehrlich (**1**, 405) thinks that in anaemia the red corpuscles break down and give rise to the structures pictured by Riess.

Laker (**1**, 192) has made this particular point the object of special study, and he comes to the conclusion that the plaques are not derived from the breaking down of the red corpuscles, for he has produced this artificially in various ways; but of all the fragments which he examined, he did not find one that he would have mistaken for a plaque. The same subject has been investigated by Schimmelbusch (**2**, 216), with similar results.

The view that the plaques are derived from the leucocytes has met with much more general acceptance, and has been defended by observers of high authority.

Riess says (**1**, 224) that they are destruction-products of the white corpuscles, produced by an insufficient nutrition of the blood. In another article (**2**, 696) he says that in pathological conditions, when the number of leucocytes is increased, we find, as a rule, that the number of plaques is increased also.

Even more to the point than the observations of Riess are those of Halla (**2**, 376–378), who has shown that while the number of plaques and leucocytes respectively is not necessarily increased or diminished at the same time, yet in general the cases where the plaques are found in the greatest abundance are those in which the leucocytes are also most numerous, or closely following a condition where they had existed in great numbers. As a matter of fact, the very cases in which we find this increase of leucocytes and plaques are generally those in which the number of red corpuscles is diminished. This fact was noticed by Riess (**2**, 696), and especially by Hayem (**11**, 120), who says that his view is supported by the results of over fifty observations. Thus we see that the points made by Riess and Halla, will serve as well to support Hayem's haematoblast theory, as the theory of the derivation of plaques from the leucocytes.

Pouchet (**2**, 136), from similarity of staining, thinks the plaques are derived from the protoplasm of the leucocytes; and Howlett, whose work was done after Hayem and Pouchet, but

about the same time as, and independently of, Bizzozero, is also of the opinion (**1**, 224) that the plaques are derived from the leucocytes, his reasons for this belief being: that they are absent from scrapings of lymphatic glands, spleen, and the medulla of bones in the dog; and that they resemble processes of leucocytes.

From the latter statement we would infer that Howlett regards the plaques as derived from the body, rather than the nuclei of the leucocyte, although he mentions that they stain like nuclei with the aniline colors.

Hayem (**4**, 49) at first appeared to advocate the view that the plaques were independent elements (éleménts particuliers, et à tous les dégrés de leur évolution, parfaitement distincts des leucocytes). In a later article (**9**, 198) he says: "The haematoblasts arise in the lymph; they are found in the protoplasm of the white corpuscles, from which they are set free before entering the blood, except in certain pathological conditions." He describes leucocytes found in the lymphatic glands, in which ready-formed plaques may be recognized. These elements possess a nucleus more or less hidden by the plaques. He does not mention the multinuclear leucocytes to which Hlava attaches so much importance, and it is possible that he had these under observation, regarding their nuclei as plaques. Löwit (**3**, 127) also has seen structures in the leucocytes, in warmed peptone blood, which he took to be plaques.

He (**3**, 125) believes that he has also seen hyaline drops exude from leucocytes, swim off in the plasma, and become plaques. He gives a figure illustrating this process, which I reproduce (Plate, Fig. 13, *c*), and from which I infer that what he has really seen is a plaque which is adhering to the edge of a leucocyte, and which has become a small bladder.[1] He has taken the hyaline part of the bladder for a plaque, while the granular part is lost against the leucocyte. Sometimes small bladders are formed, the hyaline part of which is little, if any, larger than a plaque (Plate, Figs. 11 and 12). He admits also, that this process takes place very quickly in blood drawn into salt solutions, and cannot always be seen, while it very rarely can be traced; which is well in accordance with such an inference.

Halla thinks the plaques are derived from leucocytes which

[1] See page 308.

break down in the circulation, because they are found to be most numerous at the same time, or immediately after the leucocytes are found in greatest numbers. He thinks they are nuclei of leucocytes because they resemble these structures more than the rest of the cell.

The most ardent supporter of the theory that the plaques are nuclei of leucocytes is Hlava. Hlava (**1**), after a severe criticism of Bizzozero's work, gives the results of some experiments of his own on plaques and the origin of fibrin, in which he is led to the conclusion that the plaques are nuclei of the leucocytes, which are set free when the latter break up, whether in the circulation or in extravasated blood. He adduces the following facts in support of his theory:

First (**1**, 403): He admits that he was unable to find any white corpuscle breaking down while in the circulation, but he has seen white corpuscles filled with plate-like structures. "Such white corpuscles have the greatest similarity to conglomerations of the true plaques, and stand perhaps in close relation to them."

Second: He divides the leucocytes into several varieties, and says that one definite variety of these—the multinuclear (see Plate, Fig. 14, *a*, *b*, *c*, *g*)—always breaks down much more readily than the others when the blood is shed. The total number of leucocytes is much smaller before coagulation than after, and the few which remain are nearly all of the uninuclear variety.

Third: He has observed (**1**, 404) that we find more plaques when we examine blood in salt solutions than when it is drawn into osmic acid. He gives as the reason for this that the leucocytes are set by the osmic acid, and therefore cannot break down, while a destruction of the multinuclear leucocytes takes place in salt solutions, giving rise to what have been taken for groups of plaques.

Fourth: He has also found (**1**, 408) that as the multinuclear leucocytes break down, their nuclei stain less deeply with gentian violet. The same may be noticed with regard to the breaking down of the plaques; moreover, the plaques and nuclei have about the same size and shape.

Fifth (**1**, 394): He quotes Rauschenbach that fibrin formed from a necrosis of the leucocytes has the same micro-chemical properties as the plaques; and from this he draws the conclusion

that the micro-chemical properties of the plaq[illegible] and dead leucocytes are the same.

Sixth (1, 409): He explains the presence of plaques in the blood while yet in the vessels by supposing the breaking down of the leucocytes to be a perfectly normal occurrence in the circulation. To support this view he refers to the work of Schmidt, who, with Jackowicki, Birk and others, has found that free ferment exists in the blood, and is increased in amount during fever.

In regard to the first point that Hlava makes, I can say that I have seen the corpuscle which he describes (Plate, Fig. 14, *a*, *b*, *c*, *g*), but have always regarded it simply as a multinuclear leucocyte. It is certain that dilute acetic acid brings the small bodies in them into prominence, just as with a nucleus, while there is no such reaction as that shown when the plaques are treated with this reagent. Furthermore, I have not observed any biconcavity in the small bodies within the leucocytes, such as is seen in the plaques.

As to the second observation of Hlava, I must say that my observations, in several instances where my attention was directed particularly to this point, do not go to confirm it.

In the third point Hlava is correct in stating that we find fewer plaques in osmic acid than in dilute salt solutions, but I think the reason of this is, that in salt solutions the plaques adhere to the slide or cover-slip, while the other elements are drawn hither and thither, or even washed away entirely. Thus the plaques from the whole drop of blood will collect together on the slide or cover-slip, and appear very much more numerous in proportion to the other elements than they really are. This very fact is taken advantage of to get a great many plaques under observation in the same field.

To the fourth point, I can only say, that in all the preparations of clot which I have examined I have not seen half-a-dozen leucocytes broken down, but I have seen groups of plaques which presented exactly the appearance which we would expect to find in a leucocyte breaking down; and I am only able to feel

[1] See p. 302. When the blood is mixed in proper proportions with strong salt solution, which prevents them from adhering, their number is found to be about the same as in osmic acid.

confident that it was not a leucocyte from the fact that many of the latter could be distinguished, of all varieties, which showed no signs whatever of disintegration; and, more important still, I have followed the process of breaking down in masses the individual plaques of which *I had seen come together*, and these, when well broken down, would sometimes present the appearance so easily mistaken for a leucocyte.

Hlava's fifth argument will not hold good, because by necrosis the micro-chemical properties of the leucocytes may be changed entirely. Hlava himself regards coagulation as a necrosis of the white corpuscles; but the normal plaques and leucocytes do not have the same micro-chemical properties, as I shall show later.

Hlava's sixth point also cannot be said to be free from objection. In the first place, the plaques are biconcave, while the nuclei of the leucocytes have never been made out to be so. They also differ in micro-chemical properties, the nuclei resisting the action of concentrated acetic acid longer, and also staining more readily than the plaques with certain reagents. They are not found in the blood of ovipara, nor in the blood of invertebrates, although multinuclear leucocytes are found in the blood of these animals. The fact that ferment may exist in the blood, and even be increased in fever, does not prove that the leucocytes break down, for the plaques were present also, and it may have been derived from them.

Of the older writers, Simon and Andral have mentioned structures which were possibly plaques, in connection with the formation of fibrin. (See historical section.)

The chief supporter of the view that the plaques are depositions of fibrin is Ranvier (**1**, 215 and 217), who comes to this conclusion from a few micro-chemical resemblances which exist between them, one of which is that they are both stained with iodine. Lavdovsky (**2**, 65) and Halla (**1**, 222) think that the plaques are morphologically allied to fibrin, from the intimate connection which exists between the granular masses and the fibrinous network. Ranvier also makes this a strong point.

Now, I have shown (p. 309) that iodine does not give a true stain to the plaques, and the same may be said with regard to fibrin; but in preparations stained with Bismarck brown, or methyl violet especially, we have the remnants of the plaques deeply

stained, while the fibrinous threads radiating out from them are nearly colorless, and can be seen to run directly up to the mass, from which they can be more or less sharply distinguished. The same thing may be more clearly shown if haematoxylin be used instead of Bismarck brown or methyl violet. To do this, let the plaques and fibrin stain thoroughly, then remove the excess of haematoxylin by acid. The last traces of color will be removed from the fibrin while the plaques are still deeply stained.

These methods of staining do not show, beyond doubt, micro-chemical differences between the plaques and fibrin, for in the case of haematoxylin it is only natural that the stain should be removed from such delicate structures as the fibrin-threads before it is from the granular masses. In the case of methyl violet or Bismarck brown, the difference in staining between the granular masses and the fibrin-threads seems to be too great to be accounted for by their difference in size, yet I have never been able to wash the stain entirely away from the fibrin. The action of distilled water, however, shows a true micro-chemical difference between the plaques and fibrin. The plaques take on the bladder structure described, and gradually dissolve, while the fibrin remains unchanged.

The theory that the plaques are precipitated globulins is held only by Löwit.[1] This observer believes (**3**, pp. 111, 114–117, 124–125) that the plaques are globulin depositions which at the body-temperature are normally in solution in the plasma, or contained in the leucocytes, but which are precipitated from the one by cooling, or extruded from the other when the blood is drawn. He claims to have produced plaques by precipitating separately both fibrinogen and paraglobulin (**3**, 124 and 125).

Löwit believes that the plaques do not exist in circulating blood, for the following reasons:

First (**3**, 85–93): Their number is found to vary when drawn into salt solutions of different strengths.

[1] Unfortunately, the numbers of the Sitzungsberichte of the Vienna Academy which contain Löwit's two first articles (1 and 3) were not in the University library at the time this article was written, and I was only able to find them after Schimmelbusch's later article (2) came to hand. The work of Löwit is rather out of the usual line, and I hope, in a future publication, to enter more closely upon his investigations and results than I shall be able to do here.

Second (**3**, 89): If blood be drawn into 25 per cent. salt solution, no plaques will be found.

Third (**3**, 107): The plaques in the blood of peptonized dogs are not so numerous when drawn as after standing for some time.

Fourth (**3**, 111): The plaques cannot exist in circulating blood because they dissolve in the plasma[1] at the body-temperature.

Fifth (**3**, 113): Plaques do not exist in the blood of cold-blooded animals. The element described by Hayem, Bizzozero and others, is the representative of a stage in the development of the white corpuscles.

With respect to the first reason, we should expect the number to vary in dilute salt solutions, on account of the adhesion of the plaques to the cover-slip.[2] Additional evidence in favor of the assumption that the plaques are present in the circulation, is derived from the fact that if a large drop of strong salt solution: 28 per cent. $MgSO_4$, 25 per cent. NaCl, etc., be put on the finger, and the finger pricked through the drop so as to get a small amount of blood in comparison to the drop of salt solution, the plaques are found isolated and floating freely, like the corpuscles, without showing any tendency to adhere. In this case the number of the plaques is about the same as when drawn into osmic acid.

In repeating the experiment on which Löwit bases his second reason, I can only say that although in some preparations the number of plaques was very small, I have never found a preparation in which they were entirely absent.

I have never worked with peptonized dog's blood, and therefore have no suggestions to offer, at present, in regard to the third point.

The fourth reason will be referred to with the work of Schimmelbusch.

The fifth point seems rather to be against the assumption that the plaques are globulin precipitations produced by cooling, for the blood of cold-blooded animals undoubtedly contains both paraglobulin and fibrinogen, as well as leucocytes.

Löwit grants that Bizzozero and others may have found

[1] His work was done with peptonized blood.

[2] See p. 302.

plaques in the circulating blood, but says they were produced by cooling down the mesentery. He says if the mesentery had been surrounded by .75 per cent. NaCl solution at 37°–40° C., the plaques would not have been found.

Schimmelbusch combats the views of Löwit, supporting his position mainly on two observations:

1. In repeating Löwit's experiment with normal plasma, he was unable to confirm Löwit's observations with the peptonized plasma. His method of procedure was as follows (**2**, 212):

A piece of mesentery was cut out with a red-hot scalpel, so as to prevent hemorrhage, and the plaques in the capillaries were found to be quite homogeneous. The piece of mesentery was then heated up to 40° C., but the plaques refused to dissolve.

2. He has followed Bizzozero in examining the circulation in the mesentery of the rabbit, and has found the plaques circulating in the blood through the mesenteric vessels. In this experiment (**2**, 213–217) he has taken the precaution to meet Löwit's objection by keeping the mesentery of the animal immersed in a bath of .75 per cent. salt solution at the body-temperature. The mesentery is not drawn out until the animal is in position in the bath; so that at no time is there an opportunity for a precipitate to occur on account of cooling.

The last view to be considered is that the plaques exist in the circulating blood as normal, independent elements.

That they are not due to changes produced in other elements after the blood is drawn, is shown by pricking the finger through a drop of osmic acid, by which process all the elements of the blood are set immediately upon leaving the vessel. It is also pretty conclusive proof, that four competent observers—Bizzozero (**7**, 17), Lavdovsky (**2**, 64), Hlava (**1**, 403) and Schimmelbusch (**1**, 100)—have seen them circulating through the vessels in the mesentery, and Osler (**1**, **2** and **3**) has seen them in the uninjured vessels of the connective tissue of young rats. Pouchet (**2**, 136), prior to Bizzozero, made observations which should be mentioned here as proof of the existence of free plaques in the circulation, although he regards them as derived from the leucocytes, and as being haematoblasts. His observations were made on the mesentery of rabbits. By compressing a small vessel he was able to cause stasis; by then observing the different

leucocytes he found them with plaques adhering to their surface, as well as plaques free in the plasma. He concluded that the plaques had exuded from the leucocytes; but I think, from the light of more recent investigations, we may safely explain this as we did the observations of Löwit—viz.: that the plaques are normally free in the plasma, and that they are found adhering to the leucocytes by reason of their viscosity.

There is no doubt as to the existence of the plaques in the blood, and though recognizing their close resemblances to other structures also found there, we have not as yet sufficient evidence to believe them to be other than *an independent morphological element.*

This view is held by Max Schultze (**1**, 36), Osler (**1**, 144 and **2**, 530), Bizzozero (**7**, 17 and **3**, 348), Laker (**1**, 193), Lavdovsky (**2**, 64), Halla (**2**, 378) and Schimmelbusch (**1**, 100) and (**2**, 217).

§ 5.

Histology of Coagulation with Especial Reference to the Part Played by the Plaques.

In this section it is not my intention to dwell upon the different chemical theories of the coagulation of the blood; but I shall take up the histology of the formation of fibrin, and its relation to the histological elements of the blood, the plaques in particular.

The granular masses[1] already described as a stage of the breaking down of the plaques, have been found by many observers, and various opinions are held as to their origin.[2] Schmidt (**5**, 356; 562–567) says they come from peculiar forms intermediate between the leucocytes and the red corpuscles. He calls this intermediate form "Rothe Körnerkugeln." This opinion is also supported by Semner (**1**), whose work was done in Schmidt's laboratory, and published the following year. About the same time that Semner's work came out, Schmidt (**3**, 528 and 529) described the white corpuscles as breaking down, and giving the appearance of a mass of granules, which float off singly or in small groups in the surrounding plasma. This description differs from that given by Schmidt (**3**, 562) for the

[1] See page 295.

[2] The views of the older writers may be found in the historical section.

breaking down of the "Rothe Körnerkugeln," so that he evidently makes a distinction; but he thinks that both have to do with coagulation.

The view of Schmidt and Semner is adopted by Slevogt (**1**) and Feiertag (**1**), pupils of Schmidt.

Heyl (**1**, 35–38) has observed that in Bizzozero's fluid the number of granular masses increases as the number of leucocytes decreases; also that both granular masses and leucocytes stain with methyl violet. From this evidence, together with the assumption that the leucocytes break down during coagulation, he draws the conclusion that the granular masses are derived from leucocytes.

Riess (**1**, 245) and Hlava (**1**, 404–409) both maintain that the granular masses are chiefly formed from the leucocytes, and claim to have found intermediate stages.

Osler (**2**, 531) and Laker (**1**, 148) have each given special attention to this point, and both deny most emphatically that the granular masses are derived from leucocytes.

Löwit (**1**, 281) has studied the coagulation of lymph under the microscope, and has found nothing that could be taken for leucocyte detritus.

The most generally accepted view in regard to the granular masses is that they are formed by the plaques. This view is held by Max Schultze (**1**, 36 and 37), Osler (**2**, 530), Hayem (**5**, 675 and 704 and **16**, 215), Leube (**1**, 654), Bizzozero (**3**, 352 and **7**, 18), Davidson (**1**, 1028), Laker (**1**, 183), Halla (**1**, 217), Löwit (**1**, 297), and Schimmelbusch (**1**, 101 and **2**, 220).

Hlava believes that both plaques and leucocytes form granular masses. He thinks that the plaques break down only because they are parts of white corpuscles, and has failed to observe that the plaques break down sooner than the other white elements. Löwit thinks that the granular mass proper is made up of plaques, but that leucocytes are often enclosed.

I have succeeded, in the course of my investigations, in getting preparations in which the individual plaques were seen to unite and form the granular masses. By keeping these masses under observation, I was able, as they broke down, to follow the process continuously until after fibrin was formed.

The results of my observations are given in the introductory section.

In addition to this, experiments were made, in which specimens of blood were taken at different intervals after extravasation and hardened in osmic acid, so as to get the different stages in the formation of fibrin.

The results of my work all go to show that the breaking down of the plaques is intimately connected with the formation of fibrin.

The granular masses formed by the plaques become centres from which the threads of fibrin radiate. The threads are also deposited freely in the field, and often as long, needle shaped bodies (Figs. 5, 6 and 7), but there is generally a thicker deposit of fibrin in the immediate vicinity of the granular masses, especially the large ones, than is noticeable elsewhere.

The plaques, either before or after breaking down, are not morphologically identical with fibrin, so that they do not contribute as such to the formation of the fibrinous network; the remnants which are seen enclosed by the threads of fibrin are held there mechanically, and are not an essential part of the reticulum.

That fibrin has a tendency to radiate from the granular masses as centres, has been observed by Riess (**1**, 744), Ranvier (**2**, 93), Hayem (**5**, 704 and 705), Leube (**1**, 654), Bizzozero (**7**, 19 and 20; **12**, 531), Davidson (**1**, 1028), Lavdovsky (**2**, 65) and Halla (**2**, 222).

Ranvier, Bizzozero, Davidson and Halla think there is a direct connection between the fibrin-threads and the granular masses, while Max Schultze (**1**, 38) is of the opinion that the threads pass over or through the masses, but do not proceed from them.[1] Halla (**1**, 218) has noticed that the fibrin-threads are deposited most thickly in the neighborhood of large masses of plaques. Fano (**2**, 394; **4**, 211) says that sometimes the plaques form the centres of radiation for the fibrin, but more frequently the leucocytes are such centres.

In many observations that I have made, I have found that when the plasma is very much diluted, but little fibrin is formed. Under these circumstances the field is comparatively clear, and it can then be seen that no fibrin proceeds from the leucocytes, but all comes from the plaques, or is deposited freely in the field (Plate, Fig. 6). When the clot is thicker, the fibrin is seen to

[1] From his figures one would judge the contrary.

radiate in many instances from large masses, of which it is impossible to decide positively, from their appearance, whether they are plaques or leucocytes (Plate, Figs. 5 and 7).

Sometimes a leucocyte will be covered more or less with plaques which can easily be distinguished at first, but which later would make it appear as if the leucocyte had broken down (Plate, Fig. 3). In some specimens of clot, I have seen leucocytes with only a few adherent plaques (Plate, Fig. 12). In such cases the fibrin has been seen to radiate typically from the plaques at the edge, while it bore no relation to the free margin of the leucocyte. In specimens hardened in osmic acid and stained with carmine this point can be made out very clearly. The leucocyte stains with the carmine, while the plaques and fibrin do not.

Hayem (**5**, 720) and Schimmelbusch (**2**, 242) have each pointed out that fibrin may be formed elsewhere than around the plaques. Schimmelbusch (**1**, 102; **2**, 236 and 242) believes that the fibrin does not show any preference for one or another of the histological elements of the blood. Bizzozero (**4**, 109) is of directly the opposite opinion, and states that fibrin is deposited around the plaques, and nowhere else.

When the formation of fibrin is abundant, there is a thick network of threads all over the field, and every spot is seen to be full of fibrin, whether it contains histological elements or not (Plate, Fig. 7); but when the formation of fibrin is scant, it will nearly always be noticed that the fibrin is deposited most thickly in the neighborhood of the masses of plaques (Plate I, Fig. 6). Even in preparations where the fibrin is sparingly formed, the threads are deposited elsewhere than around the granular masses, and occasionally, though rarely, I have found granular masses around which the fibrin did not appear to lie any more thickly than in the clear field. The fact that nearly always the fibrin is deposited most thickly around the granular masses, even radiating from them as centres, while interesting and significant, is not conclusive proof that the plaques are connected with coagulation; for the same adhesive property of the plaques which makes them adhere to each other, may also cause the threads of fibrin to stick fast as they separate out from the medium around them. This seems all the more probable when we consider that the fibrin as well as the plaques is sticky and adheres to the glass.

Preparations in which the clot is scanty are generally obtained from blood when diluted with a reagent which retards coagulation; and the fact that in these preparations the fibrin is deposited most thickly in the vicinity of the masses of plaques may be due to the plaques giving up something which produces or hastens the coagulation, and that in dilute solutions this substance is more plentiful in the neighborhood of the granular masses than elsewhere. More will be said of this later.

In a recent publication Laker has taken the view that the fibrin-threads are folds of a membrane which he calls the primary fibrin membrane. Laker (**2**, 156) finds that if a very thin layer of blood be gotten under the cover-slip, at the end of fifteen minutes no network will be visible, but the fibrin will be formed as a transparent membrane without differentiation. If now the cover-slip be pressed or moved, folds may be produced in the membrane which he believes to be identical with fibrin: first (**2**, 155), because their histological properties are the same, and secondly (**2**, 156), because with defibrinated blood he failed to get the membrane. There is no relation between these folds of the membrane and the cellular elements, the latter being found just as they were caught and held by the viscous substance of which the membrane is formed.

Reference has been made to such a formation of fibrin by Nasse,[1] Anderson[2] and Virchow.[3] Rauschenbach (**1**, 8 and 9) and Halla (**1**, 232) describe a membranous form of fibrin from the coagulation of fibrinogen and paraglobulin with ferment. This membrane is transparent and homogeneous, and can be plainly seen only by sundry folds in it.

The view of Laker is almost identical with that of Virchow,[3] who, as early as 1856, says: "The fibrin-coagulum is at first a homogeneous, structureless mass in which the appearance of fibres is given only by foldings on its surface or by splitting and rolling up from the edges. . . . After the coagulation is complete we have a homogeneous jelly in which lighter cells are suspended. . . . Any shaking or jarring, or movement of one

[1] Nasse, Das Blut, p. 40. Also Müller's Archiv 1841.—Quoted from Holzmann, Archiv für Anatomie und Physiologie, Phys. Abth., 1885, p. 220.

[2] Froriep's Notiz, 1844, p. 676.—Quoted from Holzmann, op. cit.

[3] Virchow, Gesammelte Abhandlung, 1856.—Quoted from Holzmann, op. cit.

part of the coagulum upon another, produces folds, which in a thick layer are confined to the surface, but in a membrane involve the whole thickness. The folds sometimes form asters and sometimes a reticulum; at other times they are parallel, but they always appear as fine, smooth lines. . . . . The cellular elements are [simply mechanically] retained by the clot."

Rindfleisch and Hermann have also mentioned a homogeneous stage in the coagulation of the blood which precedes the formation of fibrin-threads. Rindfleisch (**1**, 159) describes numerous splits and cavities which are seen as the threads of fibrin are formed by consolidation (festwerden) from the homogeneous jelly, while Hermann (**1**, Vol. 1, p. 253) thinks the threads are formed by a process closely resembling crystallization.

Schimmelbusch (**1**, 102; **2**, 236) insists very emphatically that the formation of threads of fibrin is a true crystallization process, and does not believe there is a previous stage, either homogeneous or granular. Ranvier (**1**, 217) regards the plaques as fibrinous, and says that when the blood begins to clot, processes run out from them which form the groundwork (premières travées) of the reticulum; this then develops by depositions of fibrin, just as a crystal of copper sulphate will grow in a solution of that salt.

Hayem (**5**, 719) does not believe in the fibrinous character of the plaques, but nevertheless compares the formation of fibrin to a "sort of crystallization starting from small crystals already formed."

I have often noticed the membrane which Laker has observed, and I have also noticed that folds of this membrane, when fine, cannot be distinguished from threads of fibrin; but I do not think that all fibrin-threads can be regarded as folds of such a membrane. The fibrin-threads appear to be separated out from a homogeneous mass in which they lie, but when two threads are seen to cross, they can often be seen to be distinctly marked off from each other, the one lying beneath the other, and not connected as they would be if both were folds of the same membrane. Furthermore, formations of fibrin have often been obtained in which no traces of a membrane are to be found, if such ever existed. This is generally the case in specimens of blood considerably diluted.

I think it most probable that what Laker describes as a mem-

brane is a layer of the homogeneous substance described by Virchow, Rindfleisch and Hermann, which is essentially of the same composition as fibrin, and from which the fibrin-threads are formed by a process very closely resembling crystallization, if not identical with it.

If a specimen of blood be drawn into a beaker and allowed to clot, it will first be seen to "set" into a firm jelly, in which the larger fibrin-cords develop later, and by their contraction squeeze out the serum. Now, the consolidation (festwerden) of this colloid mass into *threads*, with the formation of splits and cavities (Spalten und Lücken) between them, as described by Rindfleisch, may be looked upon as "a process closely resembling Crystallization," as Hermann defines it. The definite form which the fibrin-threads take, especially the typical needle-shaped form of the threads when deposited isolated in scanty clot, speaks strongly in favor of a crystallization; while the subsequent toughening and contraction of the threads show a clear resemblance to the coagulation of certain proteids, notably myosin.

In fact, it appears that we have in blood an interesting process which may be regarded as intermediate, in a certain sense, between a true crystallization on the one hand, and the coagulation of certain proteids, such as myosin, etc., on the other.

From the foregoing, I think it is evident that there is no histological connection between the plaques and fibrin, so that if the plaques are involved at all in coagulation, the connection must be a chemical one; that is, the plaques must give up something to the plasma at the same time that they break down.

Hayem (**5**, 704 and 705), Bizzozero (**4**, 101–103; **7**, 19), Lavdovsky (**2**, 65), Halla (**1**, 222; **2**, 378) and Ferraro (**1**, 295) have pointed out that *fibrin is formed pari passu with the breaking down of the plaques;* and both Hayem and Bizzozero have shown that *reagents or conditions which retard the breaking down of the plaques, retard to* PRECISELY THE SAME EXTENT *the formation of fibrin;* while reagents which PRESERVE THE PLAQUES PREVENT THE FORMATION OF FIBRIN ALTOGETHER.

I think the strongest evidence we have of a connection between the plaques and the clotting of the blood is derived from these facts, and all my observations thus far completed confirm them in every respect.

Schimmelbusch (**2**, 238 and 239) notices the observations of Hayem and Bizzozero, that reagents which affect the breaking down of the plaques also affect coagulation. He does not attach much importance to this, but says that the reagents employed may possibly prevent coagulation, even if the plaques broke down. He admits, however, that there is more weight to be attached to the action of cold and warmth respectively slowing or hastening the breaking down of the plaques.

Schimmelbusch (**1**, 102 and 103; **2**, 242) does not believe that there is any connection between the plaques and coagulation. The chief objections which he urges against this assumption may be given briefly as follows:

1. He refers to the experiment of Bizzozero (**2**, 308; **4**, 103), in which this observer found that in the blood of animals after death the plaques retained their original form as long as the blood remained fluid, while in blood that had clotted the plaques were found more or less broken down. Schimmelbusch (**2**, 309) made several experiments in connection with this question, and was led to deny the parallelism between the breaking down of the plaques and the clotting of the blood. He opened the hearts of cats, dogs and man, at intervals of from one to twelve hours after death, and took a small bit of fibrin, which he transferred to .75 per cent. NaCl solution, or better, to osmic acid. Upon teasing out this fibrin, he found a great number of plaques, free and intact, lying on and between the threads of fibrin. On the other hand, he found that in the hepatic vein the blood was slow to clot, but the plaques changed rapidly. The plaques, however, were star-shaped, and less distorted than in arterial blood, owing to the absence of fibrin. From these observations he draws the conclusion that there is not an inseparable connection between the breaking down of the plaques and the coagulation of the blood.

This work of Schimmelbusch is exceedingly interesting, as yielding positive results which tend to disprove the relation between the plaques and coagulation, so clearly indicated by the work of Hayem and Bizzozero. I do not think, however, that they can be regarded as *conclusive* proof to the contrary, as I hope presently to show.

To Bizzozero's observations that solutions of neutral salts

would retard the breaking down of the plaques, and also the formation of fibrin, Schimmelbusch (2, 238) objects that even if plaques broke down, the salt might still prevent coagulation by acting chemically on what was set free. Now, it seems to me that this same objection may be urged against the conclusiveness of Schimmelbusch's own observations on the hepatic blood. We know that a number of ferments and other substances can be extracted from the liver after death, and it is not impossible that some of these may act in the manner described.[1]

The fact that well-preserved plaques are found enclosed in fibrin taken from the heart some time after death, cannot be regarded as conclusive proof that the plaques are not connected with the formation of clot, unless we knew definitely what they were supposed to yield, and at the same time could be sure that the plaques are the only source from which the substance produced could be derived. We should also have to know positively that *all* the plaques were well preserved, and that *none* of them had broken down.

A striking illustration of this came up in my own work a short time ago. In a series of transfusion experiments recently made in this laboratory by Professor Martin and Mr. J. P. Campbell, peptone was tried to render the blood incoagulable. It was found to work very well for dogs, but was without effect on cats. Thinking that this might throw some light on the subject of the relation of the plaques to coagulation, I took some specimens of peptonized cat's blood for examination. The blood was drawn from the carotid artery, through a glass canula and a piece of rubber tube about six inches in length. Some specimens were taken and hardened in osmic acid with as little loss of time as possible. These were found to be the same as perfectly normal blood. What was my astonishment, however, upon examining specimens from blood which had stood about two minutes, to find a beautiful typical fibrin-reticulum, with hundreds of plaques well preserved and enclosed in the network![2] I naturally

[1] Haycraft thinks he has found such a ferment in the buccal glands of the medicinal leech, which prevents the action of fibrin-ferment. (See Proceedings of the Royal Society, London, Vol. 36, p. 478.)

[2] Preparations of peptone blood were obtained in the following manner: When it was desired to harden a specimen of blood as soon as possible after being drawn, one surface of a cover-slip was touched to the stream of blood as it flowed from

thought I had found a proof that the blood would clot without the plaques breaking down; but upon looking into the matter, I found that the tube was not clean, but that blood had been drawn through it shortly before I took my specimens, and that the walls were filled with pieces of adherent clot. Another cat was peptonized, and some specimens of blood drawn through a tube already used in the carotid, while others were drawn through a fresh tube in the femoral.

The blood drawn through the dirty tube was found to clot a little more quickly than that drawn through the clean tube. In blood drawn through the clean tube, coagulation went hand in hand with the breaking down of the plaques. From blood taken through the dirty tube, specimens could be gotten in which there was a copious formation of fibrin, but in which *nearly all* the plaques were well preserved. I could always find some broken-down plaques, but whether these were brought from the clot on the sides of the tube, or whether they broke down as the blood flowed through, of course cannot be decided definitely.

The difference in time between the clotting of blood drawn through the dirty tube, and that of the blood drawn through the clean tube, though slight, was noticeable, and this, together with the fact that when coagulation first sets in, the plaques are found preserved in the specimen from the dirty tube, while they are broken down in the specimen from the clean tube, would seem to show that *in passing through the dirty tube the blood took up something which brought about coagulation before the plaques broke down, but which is also formed later by the plaques when they break down.*

From what we know of coagulation at the present time, it seems probable that the agent in fibrin-formation that would be most apt to conduct itself in this way is the *ferment.*

Hayem (**5**, 720) and Bizzozero (**2**, 320) each think that the

the tube; a stream of .75 per cent. NaCl solution from a wash-bottle was then directed against it immediately, so as to wash off the excess of red corpuscles, and the slip was dropped into a watch-glass of 1 per cent. osmic acid.

To obtain specimens of the blood after any desired interval of time, the blood was drawn into watch-glasses and a cover-slip laid on its surface (the cover-slip will float, so that only the under surface comes in contact). At the end of the desired interval the cover-slip was lifted off with a pair of forceps, washed, and hardened as above.

part played by the plaques is to furnish something essential to the coagulation, and both agree that ferment is in all probability the agent in question. Bizzozero (**2**, 319; **4**, 111; **6**, 278; **13**, 353) attempts to prove this by his well-known experiment of whipping blood with threads, by which operation he gets them full of adherent plaques and some leucocytes. The threads are carefully washed in .75 per cent. NaCl solution, and then added to a liquid coagulable with ferment. A clot is formed sooner or later, according as there are many or few plaques adhering to the threads (**4**, 111). To eliminate the objection that the adherent leucocytes had caused the clot, as well as to disprove the theory that the leucocytes yield fibrin-ferment, he took specimens of the same coagulable liquid, and to them he added pieces of spleen, lymphatic gland, red marrow, etc., all of which contain leucocytes, and got no coagulation; from which he concludes that the leucocytes do not yield ferment, and therefore the ferment must have come from the plaques adhering to the threads.

Rauschenbach (**1**, 76–95) enters into a detailed and lengthy criticism of Bizzozero's work, in which he raises two objections to the experiment just described: First, that the threads may have absorbed ferment which was not washed out, and this it was that produced coagulation; secondly, he says that the protoplastic fluid used by Bizzozero[1] is only a test for *free* ferment. He thinks the ferment is combined in some way with the leucocytes, and that the $MgSO_4$ prevents it from being set free.

The first objection is undoubtedly valid. By granting the second, we exclude the leucocytes adhering to the threads from a share in the formation of ferment, if any is formed by what does adhere to the threads. It then remains to be explained why the time of coagulation should depend upon the number of plaques, and the experiment remains of value as offering strong support to the theory that ferment may be derived from the plaques.

[1] Bizzozero used a fluid recommended by Schmidt. It is made by drawing blood into a 28 per cent. solution of $MgSO_4$ in the proportion—blood, $MgSO_4$ :: 1 : 3. The salt solution should be at 0°. When the blood is drawn into it, a constant, but not violent, stirring is kept up until the two are well mixed. The mixture is then allowed to stand until the cellular elements have settled. The plasma is then drawn off from the top layer and filtered at 0°, and this is then the protoplastic liquid.

The question of the formation of thrombosis is not without interest as bearing upon the question of coagulation.

Before the plaques were well known, there was a great consensus of opinion among physiologists and pathologists that the "white thrombus" which is first formed around a lesion in the vascular wall was composed of leucocytes. Within the last four years this question has received the special attention of Osler (**4**; **2**, 531), Bizzozero (**7**, 19; **3**, 358; **4**, 117), Hayem (**22**, 654 and 655), Ferraro (**1**, 298) and Lubnitzky (**1**, 207), all of whom find that the white thrombus is not composed of leucocytes, as formerly supposed, but of plaques.

In connection with my work on the plaques, I have taken up the subject of thrombosis, and hope to have my results ready for publication in the near future. I may say now, however, that my work thus far completed tends to confirm the results of the later observers just mentioned, and attribute the formation of the white thrombus to an agglomeration of plaques around a lesion in the vascular wall, or a foreign body introduced into the vessel.

From the foregoing it would appear pretty evident that there is an intimate connection between the breaking down of the plaques and the coagulation of blood. On the other hand, some evidence has been adduced to show that coagulation may sometimes take place without the plaques.

Lymph will clot, and Fano and Löwit (**1**, 295) state that it contains no plaques. Hayem (**9**), on the contrary, maintains that the lymph does contain plaques, and, in fact, that the plaques in the blood have their origin in the lymph. I have not yet studied the coagulation of lymph, so that I cannot say at present whether the clotting of lymph is similar to that of blood, or whether differences exist that will throw light on the relation of the plaques to the coagulation of the blood.

Löwit (**1**, 293) also claims to have obtained specimens of blood in 25 per cent. NaCl solution in which there were no plaques, but from which he could get a clot. I have repeated this experiment of Löwit, but with different results.[1]

The strongest evidence yet brought forward against the supposition that the plaques are necessarily connected with the coagu-

[1] See p. 317.

lation of the blood, is derived from an experiment of Löwit (**1**, 297). This observer found that if rabbit's blood be mixed with 28 per cent. $MgSO_4$ solution, and allowed to stand, the whole operation being conducted at 0°, the corpuscles will soon settle, and plasma from the top layer may be obtained in which there are numerous isolated plaques, a few red corpuscles, and no leucocytes. On adding water the plasma will not clot, although the plaques in it are numerous, whereas if fibrin ferment be added it soon forms a firm jelly.

I have not had an opportunity, since consulting Löwit's article, to repeat this experiment.[1] Hayem (**5**, 708) has shown that plaques which have been subjected to temperatures of 0° C. and under for a considerable length of time, lose their power to bring about coagulation when the temperature is raised. This I have found to be true, at least for a thin film of blood and .75 per cent. NaCl solution, in an ordinary specimen for microscopic examination. It may be that the action of $MgSO_4$ is somewhat similar, and produces deep-seated chemical changes in the plaques that destroy their power to yield the agent active in producing coagulation.

From a careful consideration of both sides of the question, it is clear that much can be said for and against the theory that the plaques take part in the coagulation of the blood. But the fact that the breaking down of the plaques and coagulation go hand in hand in blood as nearly normal as it is possible to observe it, can hardly be without significance, and speaks strongly in favor of a connection between the plaques and coagulation as it normally takes place. The evidence on the other side is mostly derived from experiments involving the use of strong salt solutions and other very abnormal conditions, which detracts from its conclusiveness with regard to normal blood.

Three other methods of formation of fibrin have been given by different observers, besides those already considered:

1. From the red corpuscles.
2. From the leucocytes, the leucocytes playing the part ascribed in this paper to the plaques.
3. From the leucocytes by a process of necrosis.

Each of these will be taken up in turn.

[1] See footnote, p. 316.

The work of Virchow,[1] Hoppe-Seyler,[2] Van der Horst,[3] Heynsius,[4] Mantegazza,[5] Landois,[6] Semner,[7] Dogiel,[8] Hart[9] and Hayem (**24**, 62), all goes to show that fibrin may be obtained from the red corpuscles, and Dogiel thinks it possible that there is a relationship between the stromas of the red corpuscles and fibrinogen.

The methods employed in most of the above work place the corpuscles under conditions which do not exist in the ordinary process of coagulation; and, besides, it is now not claimed that red corpuscles are exclusively the source of fibrin, so that it is sufficient to mention this work as a matter of interest, but which need not be further discussed, as the red corpuscles do not enter the field as rivals of the plaques, in any theory of coagulation. There are two observations, however, which should be mentioned here, viz.: those of Landois and Hart.

Landois[10] and Hart[10] claim to have observed the stroma of the red corpuscles changed directly into fibrin. This was likely due to currents, as Landois admits. In speaking of the red corpuscles he says: "At first we can recognize the contour of individual corpuscles, *but as soon as a current is set up*[11] in the surrounding liquid, the masses of stromas are carried hither and thither, by which process the adhering stromas are drawn out into tough threads, the cell-contour being at the same time lost." He attributes the rapid clotting which follows the mixing of blood of animals of different species to this process.

In the case of thrombi in the vessels, Landois says: "When once the stroma-fibrin is formed, the plasma-fibrin can be deposited around it, as around a foreign body."

The next theory to be considered is, that the leucocytes play the part in coagulation which in this paper has been ascribed to the plaques.

[1] Virchow, Gesammelte Abhandlungen, 1856.
[2] Landois' Lehrbuch der Physiologie des Menschen, 4th edition, 1885, p. 57.
[3] Over de Eiwitachtige stoffen van het bloed, Leyden, 1868.
[4] Pflüger's Archiv, Vol. II, pp. 29–49; also, Vol. III, pp. 414–424.
[5] Centralblatt für die Medicinischen Wissenschaften, 1868, p. 292.
[6] Centralblatt für die Medicinischen Wissenschaften, 1874, pp. 420–422.
[7] Ueber die Faserstoffbildung im Amphibien und Vogelblut, etc., Dorpat, 1874.
[8] Centralblatt für die Medicinischen Wissenschaften, 1875.
[9] Quarterly Journal Microscopical Science, 1882, p. 255.
[10] Op. cit.
[11] The italics are mine.

The belief that the leucocytes break down during coagulation, thus furnishing ferment, has always gone hand in hand with Schmidt's theory of the origin of fibrin from three fibrin factors; and as Schmidt's theory for a long time met with almost universal acceptance, it is natural that the theory of the breaking down of the leucocytes should have gained a firm hold on the minds of physiologists and pathologists. It is therefore not strange that often in work on subjects involving the question of coagulation of the blood, the leucocytes have been said to break down, not because the attention of the observer was directed to this point, but because it was a generally accepted fact. This will account for a large amount of literature that may be quoted apparently in support of the theory of the breaking down of the leucocytes as part of coagulation.

This question has been the subject of many researches, however, and has been approached in many different ways, with almost as many varying results.

Schmidt, Hoffman and Heyl have found that there were fewer leucocytes in blood after coagulation than before, and from this draw the conclusion that the leucocytes break down during the process. Heyl says there is a loss of 70 per cent. of the leucocytes, while Schmidt estimates the loss at 90 per cent.

These results were obtained for defibrinated blood, and the obvious objection has been raised by Bizzozero, Laker and others that whipping the blood would injure the leucocytes, and hence these figures would not hold good for normal clot. Heyl (**1**, 30) himself recognizes the fact that leucocytes are destroyed by whipping, for he shows that if blood be shaken instead of whipped, the loss of leucocytes is considerably smaller. He also points out that by shaking a solution containing fibrinogen, paraglobulin and fibrin-ferment, the time of coagulation is not affected, while if the same be tried with blood, it hastens coagulation. He therefore concludes that the leucocytes are broken down by shaking, and that the reduced time of clotting is due to this.

These observations of Heyl show pretty clearly that numerical results obtained from blood which has been shaken or whipped for defibrination, will not hold good for blood under normal conditions. It is obvious that Heyl's observation on the acceleration

of clotting by shaking does not prove that the *leucocytes* have anything to do with it, for he had the plaques and the leucocytes together in the blood, and from the extreme vulnerability of the plaques, we should expect *them* to be broken down by shaking or whipping. This objection may be raised to the work of Feiertag (**1**), who made comparative observations on the number of leucocytes and plaques before and after coagulation. To prove a connection between the leucocytes and coagulation, Hlava (**1**, 396 and 397) refers to work done by Heyl (**1**, 47), who, following v. Samson Himmelstjerna and Hoffman, injected septic matter into the veins of animals, and studied the relation of the increased number of leucocytes to the yield of fibrin. It is quite evident that these results throw no light on the subject, as here also the plaques as well as the leucocytes were involved. Laker makes a good point also: that if 71 per cent. of the leucocytes really did break down during coagulation, *some* of them should be caught in the act. Schmidt (**3**, 528) says that he has found all stages in the breaking down of the leucocytes, but admits that he has never seen progressive changes in one individual, while Rauschenbach compares the breaking down of the leucocytes to an explosion (Der Vorgang verlauft . . . . in fast explosiver Weise.)—a view also adopted by Groth (**1**, 69 and 70).

Hlava (**1**, 410) says he has seen leucocytes arrested in different stages of disintegration, but like Schmidt does not claim to have observed changes in the same leucocyte.

A view directly opposite to this is taken by Bizzozero (**7**, 20), Laker (**1**, 198), Lavdovsky (**2**, 65) and Löwit (**1**, 277, 280–283, 302). Bizzozero and Laker are of the opinion that the leucocytes undergo no change whatever during coagulation. Lavdovsky says, "The breaking down of the leucocytes, if it occurs at all, is of no importance in clotting, and can scarcely be perceived."

Bizzozero, Laker and Löwit have all seen the leucocytes in blood after coagulation, and Bizzozero and Laker have kept leucocytes under observation for several days without finding any disintegration. My experience has been the same. Hlava (**1**, 408, 409, 416) and Rauschenbach (**1**, 12 and 13) have advanced a theory, based on their direct observations, which will account for this—viz.: that there are different varieties of leucocytes, and that one variety breaks down readily, while others do not.

My observations on this point have already been given (pp. 313, 314), and, as is seen, do not confirm it. The results of my work on this subject receive confirmation from the work of Löwit on the lymph. Löwit has counted the leucocytes in a given field before and after coagulation, and finds their number to be the same. Löwit thinks that the leucocytes participate in coagulation by emitting something which dissolves in the plasma; but from work both on blood and lymph, he comes to the conclusion that they do not break down.

This view, according to Bizzozero (**4**, 99), was first held by Mantegazza (1871), and I have seen no mention of any other advocate of it up to the time of Löwit.

The following observations of Löwit have led him to the belief that the leucocytes take part in coagulation:

He has found (**1**, 290) that if lymph containing leucocytes be added to hydrocele or ascites-fluid, it will cause coagulation. If, however, the lymph had been previously filtered through glass wool, and the leucocytes thus removed without breaking down, no clot would result from the addition of the lymph-plasma.

He claims (**1**, 293) to get blood in which there are no plaques by drawing it into 25 per cent. NaCl solution. On diluting this mixture it will clot if leucocytes be present, otherwise not.

He has observed (**1**, 301) that if blood be drawn into vessels surrounded with ice, and the different histological elements allowed to settle, they will be found in layers, some of which are particularly rich in one kind of corpuscles, some in another. If now a portion be taken from the layer which is richest in leucocytes, it will be found to produce coagulation more readily than specimens from layers poorer in leucocytes. These results are very striking, and would almost carry conviction on their face, but Löwit also mentions (**1**, 300) that the layer in which leucocytes are most plentiful also contains many masses of plaques, which, of course, detracts from the decisiveness of the experiment.

Rauschenbach has succeeded in extracting ferment from leucocytes obtained from a number of sources, but his methods involved rubbing them up into a pulp, treating them with distilled water, salt solutions, the serum of blood from species other than their own, etc. These results are all interesting and suggestive, but do not show that ferment is derived from the

leucocytes as a normal part of the coagulation. It is well known that peptone injected into the veins of some animals prevents the blood from clotting. Such blood has been studied for information regarding coagulation chiefly by Fano and Wooldridge, and many points of great interest are suggested by their work. I may say, however, that from this work there has been derived no positive proof that the leucocytes furnish ferment in ordinary coagulation. The ferment has always been obtained by the action of distilled water, a continued stream of carbon dioxide, etc.—conditions which are not present in the normal clotting of the blood.

There is great need of more thorough microscopic investigation combined with the chemical; and as I propose, in continuing my work on coagulation, to take up a series of investigations in this field, I shall leave a detailed discussion of this subject for a future publication.

We are now in a position to decide between the relative claims of the plaques and of the leucocytes to the function of yielding ferment, or being otherwise concerned in the coagulation of the blood. It can by no means be said that it is *impossible* to get ferment from the leucocytes. The results of many observers are strongly in favor of the assumption that, under certain circumstances, the ferment can be so derived. Rauschenbach (**1**), Wooldridge (**6**, 417), Lea Green[1] and Holzmann,[2] claim to have extracted ferment from sources other than the cellular elements of the blood. Bizzozero (**2**, 321), while claiming for the plaques the power of furnishing ferment, expressly states that he does not attribute the origin of ferment exclusively to these elements. The point which I wish to emphasize is this: that whatever may occur in leucocytes subjected to abnormal conditions—from chemical reagents, crushing, concussion, from whipping of the blood, etc.—in the coagulation of the blood *as it normally occurs* there is no *histological* evidence that the leucocytes take part, while the contrary is true of the plaques.

I may here add that I have seen apparently abnormal leucocytes in many specimens of clot. I do not, however, regard this as proof that the leucocytes break down during coagulation, but

[1] Journal of Physiology, Vol. IV, pp. 380–386.

[2] Holzmann, op. cit.

think it more likely that the leucocyte was in that condition when the blood was drawn, or that, possibly, alterations were produced by its being caught in the contracting fibrin-threads. In specimens where the coagulation was studied in Bizzozero's fluid, or at a reduced temperature, where the *process is so retarded that its individual steps can be distinguished*, I have never seen anything that could be taken for PROGRESSIVE CHANGES *in the* LEUCOCYTES, *while such changes in the* PLAQUES *are most striking*.

The last theory to be considered is that fibrin is formed by a necrosis of the white corpuscles.

This view is foreshadowed in Beale (**1** and **2**), and was next taken up by Weigert, whose object was to point out the interesting generalization that a necrosis of structured cells produced a substance resembling fibrin. He would gladly include blood-fibrin in this generalization, deriving it from a necrosis of the leucocytes, but he is forced to admit (**2**, 92) that "Fibringerinnung" is "ganz isolirt," and to speak (**1**, 469) of the substance derived from the leucocytes as "fibrinähnlich."

The later supporters of this view are Wooldridge and Hlava.

Wooldridge believes that in ordinary coagulation of the blood there are two processes, one of which involves substances in solution, the other the cell-substance of the leucocytes. His view is briefly given by himself (**2**, 418) as follows:

"There are two essential processes in the coagulation of the blood, one of which has been, hitherto, entirely wrongly appreciated or overlooked. This latter process is that the 'dead' plasma converts the white corpuscles directly into fibrin. At the same time, however, that this occurs, a substance is liberated from the cells which converts the fibrinogen also into fibrin. This is the other process. The substance which is liberated from the cells is fibrin-ferment."

The view of Hlava (**1**, 414) is very similar to that of Wooldridge. He believes that fibrin is produced directly from a necrosis of the leucocytes. For convenience of description he divides the formation of fibrin into four stages:

1. The agglomeration of the leucocytes.

2. The breaking down of these (the protoplasm breaking down before the nuclei).

3. The death of the nuclei (which according to Hlava are plaques).

4. The solidification of the fibrin.

At the third stage the ferment is set free, which causes the coagulation of the fibrinogen and paraglobulin. The fibrin is first granular, then fibrillar.

I think the most likely explanation of these results has already been given—viz.: that groups of plaques may have been mistaken for leucocytes, and that in many experiments he used a concentrated solution of mercuric chloride, which produces a precipitate of proteids in the plasma that obscures the whole field and vitiates the accuracy of any observations.

Rauschenbach (1, 52) has given attention to the formation of fibrin in the presence of many leucocytes, and his conclusion is that the fibrin formed by adding different kinds of leucocytes to plasma is true fibrin produced by the spontaneous coagulation of the plasma, the apparent differences being due only to the detritus of the leucocytes, and other foreign matter mechanically retained.

SUMMARY.

The conclusions arrived at in this paper may be given briefly, in summary, as follows:

1. In addition to the red corpuscles and leucocytes, the blood normally contains a third histological element, the *plaques*.

2. Although strong resemblances exist between the plaques and other histological elements of the blood, there is not yet sufficient evidence to establish a *genetic* connection. We are therefore obliged, for the present at least, to regard the plaques as independent elements.

3. When the blood is drawn, the plaques break down almost immediately. This is not true of any other element of the blood.

4. The breaking down of the plaques is intimately connected, in its time-relations at least, with the clotting of the blood.

5. The connection between the breaking down of the plaques and the coagulation of the blood is not histological, but chemical—*i. e.*, the plaques appear to give up a soluble substance which is active in coagulation.

6. The active agent in question is most probably *fibrin-ferment.*

7. Fibrin is deposited histologically independent of any of the cellular elements of the blood.

8. When the clot is very scant, fibrin is deposited as long, needle-shaped, crystal-like bodies.

In conclusion, I would express my thanks to Prof. Martin for encouragement and valuable suggestions throughout my work, and also to Dr. J. R. Duggan, who kindly assisted me in photographing the plaques, and to whose skill and experience in micro-photography I feel my success in this direction has largely been due.

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The numbering of the articles does not in every case correspond with the order in which they appeared, but as an attempt to rearrange the numbers so as to get the articles in the proper order of publication would involve not only considerable labor, but also a great risk of getting the references confused, I have thought it advisable not to make any change.

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DESCRIPTION OF PHOTOGRAPH.

The photograph was taken from a specimen of dog's blood prepared as follows: A cannula was introduced into the femoral artery and the blood allowed to stream freely through. A cover-slip was then held in a pair of forceps and touched to the stream of blood; without losing a moment a stream of .75 per cent. NaCl solution was directed obliquely against the slip, which was then instantly dropped into a watch-glass of 1 per cent. arsenic acid. After about twenty

minutes it was taken out, washed and left all night in a 1 per cent. aqueous solution of Bismarck brown. It was then washed in water and mounted in a saturated solution of potassium acetate.

The photograph is of especial interest as being the first ever obtained, and showing at once the following points:

1. The form of the plaques (circular and oval).
2. Their biconcavity (appearing as a light shade in the centre of the plaque).
3. Their variation in size.
4. Their tendency to adhere to each other and to the cover-slip within a few seconds. Their adherence to the cover-slip is shown by the red corpuscles in the field. These appear as circular discs with a bright centre and a halo around their periphery. This is due to their being out of focus, the objective being focussed on the plaques adhering to the slip, while the red corpuscles lie in the liquid between the slip and the slide.
5. They are not fragments of disintegrated leucocytes, but are distinct histological elements.

DESCRIPTION OF FIGURES, PLATE XIX.

FIGURE 1.—Drawn from an unstained specimen prepared as described for photograph. The lenses used were Zeiss $\frac{1}{18}$ homogeneous immersion obj., oc. 4 (x = 1450), with Abbe condenser. *a*, Plaques seen full on face, slightly or not at all altered. *a'*, Plaques slightly altered in outline, showing the first changes toward breaking down. *b*, Plaque seen on edge. *b'*, Plaque seen partly on edge and partly on surface. *c*, Group of three plaques, each seen in different position. *d*, Plaque fixed at one end and drawn out by currents. This is quite a common appearance. The large, round figure to the right is a red corpuscle drawn to scale for comparison in size with the plaques.

FIG. 2.—Group of plaques enclosing three red corpuscles. The plaques have now become granular and of irregular outline. This is the stage in which the plaques are most commonly found when examined in .75 per cent. NaCl solution or Bizzozero's fluid. Drawn with Zeiss $\frac{1}{18}$ homogen. im., oc. 2 (x = 790), with Abbe condenser.

FIG. 3.—Same as Fig. 2, except that the plaques are more broken down, and farther on the way to form a typical granular mass. It will be observed that the plaques are not only jagged and granular, but they have begun to fuse together and lose their individual out-

line. The group includes a red corpuscle and a tetranuclear leucocyte. Lenses same as in Fig. 2.

FIG. 4.—Typical granular mass. The plaques are so far fused together that, for the most part, their individual identity is lost. Lenses same as Figs. 2 and 3.

FIG. 5.—Typical granular mass with fibrin-threads radiating from it, and needle-shaped threads (crystalloids) deposited freely in the field.

FIG. 6.—Drawn from specimen of scanty clot in diluted blood. Shows how fibrin is deposited freely in the field, as well as in connection with the granular masses.

FIG. 7.—Thick formation of fibrin. Shows fibrin-threads branching all over the field, enclosing granular masses, red corpuscles and a trinuclear leucocyte. Drawn from a specimen stained with methyl violet.

FIG. 8.—Single plaques showing the "bladder formation" from the action of water. The granules seen in the light part of the "bladders" are due to the method of preparing the plate. The dark part should be granular, as represented; the light part should be perfectly homogeneous.

FIGS. 9 and 10.—Plaques which had come together and afterwards swollen up from the action of iodine solution (aqueous). The peculiar shape was likely produced by pressure on the cover-glass.

FIG. 11.—Same as Figs. 9 and 10. Drawn particularly for comparison with Fig. 13, *q. v.*

FIG. 12.—Leucocyte with adherent plaques which have swollen up and formed "bladders." From a specimen stained with methyl violet.

FIG. 13.—After Löwit. Löwit gives the figure (especially *c*) to illustrate the extrusion of plaques from the body of leucocytes, and I reproduce his drawing to compare with Figs. 11 and 12. (See text.)

FIG. 14.—Leucocytes. *a*, *b*, *c* [and *g* (?)], multinuclear leucocytes supposed by Rauschenbach and Hlava to break down during coagulation, while the uninuclear variety *d* do not break down. Notice the resemblance of the smaller nuclei in *a*, *b*, *c* and *g* to the plaques in Fig. 2.

LIFE HISTORY OF THALASSEMA. By H. W. CONN, Instructor in Biology at Wesleyan University. With Plates XX, XXI, XXII and XXIII.

The relation of Gephyreans to other groups of worms is a question which has undergone considerable discussion, and one in regard to which there has been much difference of opinion. Indeed it has been questioned whether they are worms at all; not a few naturalists having regarded them as related to the Echinoderms. They have been looked upon as having affinities with the Turbellarian worms and the Rotifers. Within a few years, however, it has been recognized that they are to be classed with the Annelids. At least this is true in regard to *Gephyrea armata*, including Echiurus, Thalassema and Bonellia. The relation of the adult to the annelid has been for some time recognized, but it remained for Hatschek[1] to show that their development was entirely upon the annelid type. Our knowledge of the development of the group has been, however, rather meager, and is confined entirely to the later stages. Kowalevsky[2] indeed states that Thalassema has a regular segmentation and an invaginate gastrula, but beyond this nothing of the early stages is known. The observations on the early stages here to be described are supplementary to those already made and fill up a gap which existed in our knowledge of the embryology of the group. With these observations we have a nearly complete history of Thalassema and Echiurus from the first appearance of the ova and spermatozoa in the peritoneal lining of the body-cavity, to the adult individual.

The observations here embodied were made at the marine laboratory of the Johns Hopkins University, at Beaufort, being completed during the summer of 1884. The Thalassema found at Beaufort is an undescribed species, to which I have given the name *T. mellita*. It is of a dull red color, with a light yellow

[1] Hatschek. Ueber Entwick. von Echiurus. Arb. a. d. Zool. Inst. Wien, III.
[2] Kowalevsky. Zeit. f. wiss. Zool. XXII, p. 284.

pre-oral lobe. A full-grown adult reaches the length of one inch exclusive of the pre-oral lobe, which may be expanded several inches. The skin is nearly smooth, but near the anus is roughened by some minute whitish papillae. Eight light bands can be seen extending from one end of the body to the other, which are due to local thickenings of the muscles of the body-wall. This species has the peculiar habit of seizing empty sand-dollar shells (mellita) and making its home in them. It enters the shell at the oral opening while yet very small, but once within its house it grows to its adult size, and is obliged therefore to remain during the rest of its life a prisoner. One fact about this habitation, very convenient to the collector, is that each inhabited shell is marked in a peculiar manner. Directly over the animal is seen a reddish-brown horseshoe-shaped mark, which makes it very conspicuous and enables one to select at a glance all inhabited shells. Occasionally I have found specimens in cavities in other shells, having found one or two individuals in artificial cavities in old corroded oyster-shells. As far as I am aware, however, the species always inhabits some such cavity, for I have never found any living free.

Embryos may be obtained in quantities by artificial fertilization. The sexual pouches are filled during the whole summer with sexual products always in a mature condition, and fertilization takes place without difficulty by simply mixing the ova and spermatozoa. My observations have been carried on chiefly upon living specimens. To make out certain features, however, stained specimens and sections were resorted to. Owing to the very minute size it was very difficult to manipulate the embryos, and my sections have therefore not been as satisfactory as I could wish.

I.—*Origin of Sexual Products.*

The two sexes of Thalassema are separate, but with no external mark to distinguish them. A practised eye can, however, distinguish one filled with ova from one filled with spermatozoa, and can thus during the breeding season separate the sexes. The males and females living solitary, and with no power of locomotion, cannot unite, and the fertilization of the ova is therefore due to chance. Ova and spermatozoa are dis-

charged into the water, and depend upon currents for their distribution and consequent fertilization; and we consequently find both ova and spermatozoa present in great quantities. Upon opening an individual in the breeding season we find lying among the folds of the alimentary canal, four long white pouches attached to the body-wall just behind the ventral setae. In the present species of Thalassema there are two pairs of such pouches, and the same is true of *T. barronii*, *T. neptunii*, *T. gallasii*. In a species studied by Kowalevsky,[1] and in *T. moebii* three pairs are found. These pouches are receptacles for storing away the ripe ova and spermatozoa until they are to be ejected from the body. They are, therefore, secondary sexual organs, but have no connection whatever with the true sexual organs which give rise to the ova and spermatozoa.

The true sexual organs are found in the posterior part of the body near the anus, and are simply particularly differentiated parts of the general peritoneal lining of the body-cavity. Stretching through the body-cavity from the intestine to the body-wall in every direction are numerous muscular bands. One of these muscular bands, about one-eighth inch in front of the anus, extends from the intestine to the ventral nervous chord, and is considerably broader than any of the others. It is on the sides of this band and from the peritoneal cells which cover it that the sexual products are produced. During the breeding season, which lasts for a number of months, this muscular band, which serves as ovary or testes, as the case may be, is seen to be covered continually with a multitude of spherical cells, which are very abundant and very prominent. There is not very much difference in the appearance of ovary and testes, even to the microscope, but a careful study will show the distinct, though very minute ova (Fig. 47) which serve to distinguish the ovary from the testes (Fig. 44). Here then is where the ova and spermatozoa first appear as modified peritoneal cells; always, however, very small and immature. In no case do they go through any great part of their development in this position.

Upon examining a section of the ovary we find two different bodies clinging to the side of the muscular band (Fig. 47). The

[1] Kowalevsky. Z. f. w. Z. XXII.

first are the rudimentary ova *ov*, consisting of a clear body and a granular nucleus; second, there are a large number of granular bodies deeply staining with haematoxylin, and bearing a very close resemblance to the nuclei of the ova, except that they are somewhat smaller, *n*. What may be their significance it is hard to say. We are hardly justified in concluding that they are the nuclei of such peritoneal cells as are not destined to become ova, although this seems the most ready explanation. All of the ova are very small, hardly larger than the individual peritoneal cells; from which, however, they are readily distinguished by their clear round body and prominent nucleus. As soon, however, as the ova become so distinct that they can be distinguished as ova, they break away from the ovary in masses. A dozen or more minute ova, together with numerous nuclear bodies, lose their connection with the muscular band, and clinging together in clusters, become free in the body-cavity. The rest of the growth until maturity takes place while these clusters of germinal cells float freely in the body-cavity. A microscopic examination of the peri-visceral fluid during the breeding season shows that the body-cavity is filled from one extremity to the other with these masses of ova in different stages of growth (Fig. 48). The ova of any one cluster are not in any one stage of growth, one or two quite large ones being always associated with many smaller ones. The ova now rapidly grow, and just before reaching full size each one becomes detached from the rest of the cluster with which it has been associated, and floats around alone, until it reaches its full growth, when it is stowed away in one of the sexual pouches.

The growth of the ovum is illustrated in Figs. 47–50. The body of the cell, consisting of clear protoplasm, enlarges (Fig. 48 *ov''*). The nucleus is, however, most changed. It increases much in size, and the granular contents which is originally present expands with its growth, until finally it is seen as a fine reticulum dispersed through the otherwise perfectly clear germinal vesicle (Fig. 50). The germinal spot hardly changes its appearance from the first, the only change being a slight increase in size. For some time the ovum remains clear as at first, but after reaching about half its full size it begins to store up food for the future embryo in the form of yolk-granules, and soon

becomes completely filled with them (Figs. 49 and 50). There now appears in connection with the formation of a vitelline membrane which is excreted from the egg, a very remarkable structure which I am at a loss to understand. I have endeavored to represent it in Fig. 49, a highly magnified section of a portion of the ovum near its surface. The appearance was in hardened specimens that of a dense covering of cilia. Indeed, Cosmovici[1] describes the ovum of Phascolosoma as ciliated, and it is quite probable that what he saw was the same as here figured. When the eggs are examined alive, however, it is seen that it is really not cilia, for there is not the slightest movement to be seen, but the whole vitelline membrane is covered with great numbers of exceedingly fine processes. What may be the significance of this peculiar structure it is difficult to say. It is not seen in the young ova, nor in the masses of ova fully developed and stored in the sexual pouches. A suggestion as to its function which appears quite probable will appear farther on. After the development of this striated layer, the ovum soon finds its way into the sexual pouches, and is here stored for some time. We thus see that in the female Thalassema single cells of the peritoneal lining of the body-cavity take on the function of ova, and each cell grows into one egg and no more.

With the male sexual product the development is somewhat different. Here too the peritoneal cells are the sexual cells, and here too the muscular band extending from the intestine to the ventral chord serves as the place of origin of the sexual products (Fig. 44). The peritoneal cells become quite large and perfectly spherical, and lie in thick masses on the sides of the muscular band. They also soon break away from their place of origin and complete their development, floating freely in the body-cavity. Each of the germinal cells, however, proves to be the mother of a number of spermatozoa. The first indication of this is the appearance of small granules within the cell (Fig. 45), which are the bodies of the future spermatozoa. They grow more and more distinct, and finally each body develops a tail and the spermatozoon is complete. They still cling together in a curious fashion. The original cluster of a dozen or so germinal cells evidently gives rise to a great many individual spermatozoa,

[1] An. and Mag. Nat. Hist., Ser. V, Vol. IV, p. 95.

but the whole of them cling together by their heads (Fig. 46), even after they are fully formed and the individual mother-cells can no longer be distinguished. With the movements of the tails they are driven hither and thither, and are found in all parts of the body from pre-oral lobe to the anus, but always persistently clinging together. Finally, just before entering the sexual pouches, they are arranged into irregular hollow spheres with their tails pointing outward. When once within these pouches, however, they immediately break up and the spermatozoa are afterwards entirely separate.

The most interesting point connected with the development of ova and spermatozoa is the exceedingly simple method of their growth. From the time when the ovum separates from the place of its origin it has no further vital connection with the adult, but grows through the rest of its development as an independent organism. It grows many times its original bulk (compare Fig. 47*ov'* with Fig. 50), and all of this nutritive material must be obtained from the peri-visceral blood. We see in this point evidence that the peri-visceral fluid is highly important in the circulation of nutriment; and we see further, that each ovum is an independent organism from the very first. From the time when they break away from the ovary, the ova have no vital connection with the mother, yet they are able to pick up large quantities of food from the fluid in which they float, and stow it away in the form of granules. They develop a large germinal vesicle with a germinal spot, and finally secrete the vitelline membrane and the above-mentioned striated layer. We thus see that even when they break away from their place of origin, although very immature, they are yet capable of independent existence under favorable circumstances. They are in fact even now independent individuals.

Having completed their development in the body-cavity, the ova and spermatozoa are finally collected in the sexual pouches. In regard to these pouches there has been in the history of Gephyrea a considerable difference of opinion. They have been considered generative sacs,[1] testes,[2] and again are called uteri.[3] Quatrefages showed them to be temporary reservoirs for the

[1] Forbes and Goodsir, *loc. cit.*
[2] Quatrefages. An. d. Sci. Nat., Ser. III, t. vii.
[3] Lacaze-Duthiers. An. d. Sci. Nat., Ser. III, t. x.

sexual products. Spengel[1] and Greeff[2] have tried to show that they are homologues of segmental organs, a homology which is probably a correct one.

Each of these pouches is a somewhat muscular, very extensible sac, varying greatly in its appearance at different seasons of the year, being in the summer distended with the sexual products, and in the winter empty. Their walls are composed of two layers of muscles lined by epithelial, and covered on the outside by the general peritoneal lining of the body-cavity (Fig. 51). Each sac has two openings, one through the muscles of the body-wall to the exterior *e. o.*, and the other into the body-cavity. The latter opening is by means of a long tube which is guarded above by a process of the sac (Fig. 51, *u. l.*), and below by a process from the body-wall, *l. l.* The whole forms a little cup-like structure at the base of the large sac. It is through this opening that the sexual products find their way from the body-cavity into the pouches. The canal is lined with cilia (Fig. 51), which, in an almost intelligent manner, pick up the ova and spermatozoa, separating them completely from the numerous blood corpuscles and driving them into the sacs. I was for a long time puzzled to imagine how this selection could take place since the spermatozoa are smaller and the ova larger than the blood corpuscles, but after making out the development of the ova and spermatozoa an explanation suggested itself. It is evident in the first place that no current of the blood can be created by the cilia entering the sexual sacs, since they are closed at their inner extremities. They are like distended india-rubber sacs in which no more fluid can be forced. But the cilia might be able to pick up solid particles and force them in. Now we have seen that when the ovum gets ready to enter this pouch it is covered by a peculiar fuzzy layer, and this layer would evidently enable the cilia in the tube (Fig. 51) to obtain firm enough hold of it to force it into the pouch. The spermatozoa, we have also seen, when fully grown, also are arranged together in masses with the long tails projecting outward, and these tails would serve the same function as the covering of the ovum. On the other hand, the blood corpuscle is much smaller than the ovum or the mass of

[1] Mitt. a. d. Zool. Sta. in Neaples, Bd. I.

[2] Greeff. Sitz. d. Ges. z. Bef. d. Ges. Naturw. zu Marburg, 1879, No. 4.

spermatozoa, and is moreover smooth. It cannot therefore be seized directly by the cilia; and since no current can be established flowing inward, there is nothing to drive the corpuscle into the sac. Of the various bodies which reach the mouth of the tube, the large ones, which are covered with numerous projecting processes, can be grasped by the cilia; while the rejection of the smaller blood corpuscles is not intelligent, but simply a mechanical impossibility of their being seized: certainly this is a very pretty contrivance.

When once within the sexual sac the ova and spermatozoa do not immediately pass out of the external opening, as seems to be the case in Echiurus, but they remain here in a dormant condition for a long time. All four of the sexual pouches are frequently found distended with ova, which are crowded very compactly together. Knowing what we do of the moderately slow development of the ova, it is evident that it must have taken a long time—weeks, and perhaps months—to have brought to maturity the thousands of ova which are thus collected together. Indeed, during the whole time of my experiments from June until September, it was a very rare thing to find an individual without its sexual pouches distended with ova or spermatozoa; and they are always, of course, mature, the ova capable of immediate fertilization, and the spermatozoa always ready to fertilize the egg. It is rather surprising that they should retain their vitality for so long a time, all the time mature and ready to develop immediately upon being brought in contact with each other; but it is certainly the case.

I have never seen the discharge of the ova from the animal, and do not know how it is accomplished, except that they must be ejected through the external opening (Fig. 51) with which each pouch communicates with the exterior. Nor am I able to say with certainty that the sexual pouches discharge all of their contents at once. I have kept the animals in aquaria for a long time and they have not discharged a single ovum. From the fact that in almost every case the pouches are during the summer filled with ova, and from the fact that the ova are always mature, I come to the conclusion that as the ova and spermatozoa reach maturity they are stowed away and remain in the pouches until they are filled, when the entire contents are discharged at once.

II.—*Polar Globules and Segmentation.*

The ovum of Thalassema is very small, being no more than $\frac{1}{350}$ of an inch in diameter. When first removed from the genital pouches, where they have been stored for a long time, they present the appearance of Fig. 1. Being crowded closely together in the pouches, they are never spherical, being more or less indented by the surrounding ova. In surface view very little can be seen, owing to the multitudes of yolk-globules with which each ovum is crowded. A light spot in the center indicates the position of the nucleus or germinal vesicle. A section of the ovum, however, shows its structure more completely. Such sections show the ovum to have a very thin vitelline membrane closely applied to the egg proper (Fig. 50). In the center of the egg is the large nucleus and an enclosed nucleolus. The nucleus is filled by a fine protoplasmic network, and is bounded by a very distinct and quite resisting membrane. This nuclear membrane of the unfertilized egg is a very prominent structure, as is shown both by section and by its behavior under compression; a marked contrast to the segmentation nucleus, which is the result of fertilization, and is devoid of any membrane.

The only point of any particular interest in regard to this ovum is its compressed shape, and the fact that under the influence of sea-water alone it will *not* assume a spherical form. The eggs are heavier than water and soon sink. If left now without fertilization, nothing further will happen to them except decomposition.

Quite different is the history of the egg if it be fertilized. Within one minute and a half after the entrance of a spermatozoon into the egg a complete change takes place. I have found it impossible, on account of the opacity of the ovum, to follow very minutely the changes accompanying fertilization, but the points it was possible to make out were as follows: Immediately upon the entrance of the spermatozoon into the egg it exercises some remarkable influence upon it. Fertilization proper does not take place, as we shall see, until some half an hour later, but the entrance of the spermatozoon calls into activity powers which were lying dormant hitherto. As soon as the first spermatozoon enters the egg, the egg begins to swell

rapidly, and in about two minutes it has become perfectly spherical. This can be considered due to the absorption of water, since the solid contents cannot of course increase. What becomes of the spermatozoon I have found it impossible to make out, but judging from what happens in other cases which have been studied, it is probable that it remains entirely inactive during the changes which now follow. The germinal vesicle now disappears entirely, as far at least as external appearances go. A careful study indicates, partially at least, to what this disappearance is due. Previously, as shown, it consists of a large, approximately spherical vesicle with a definite membrane, and containing a clear substance, and entirely devoid of yolk-spherules (Fig. 50). Now, under the influence of the active changes, the vesicular membrane entirely disappears, becoming absorbed, and, as a consequence, the yolk-globules migrate into the space previously occupied by the vesicle. As soon as this occurs all indication of the germinal vesicle disappears, and the egg appears as an opaque sphere.

Meantime a second noteworthy change has taken place. In the unfertilized egg the vitelline membrane is very closely applied to the egg contents, so closely in fact that it can be distinguished only in section; but in the course of about five minutes after the entrance of the spermatozoon the egg appears to contract slightly, and to shrink away from the vitelline membrane. The result is that there appears quite a noticeable space between the egg and the membrane, a space seemingly filled with a perfectly transparent liquid of some sort. This drawing away of the egg from the membrane is usually explained as a contrivance to prevent the entrance of other spermatozoa, and it would certainly have this effect if it occurred at the proper time. But in this instance it hardly seems possible that such can be its function. In the first place it does not occur until five minutes after the entrance of the spermatozoon, and there is in the meantime ample chance for other spermatozoa to make entrance; and secondly, in all the cases of artificial impregnation which have come under my observation there were hundreds of spermatozoa clinging to the egg long before this defensive change took place. All of these cases, as well as a few in which only a single spermatozoon was allowed to come

in contact with the egg, develop in a perfectly normal manner, and as far as can be made out from external appearances there is no difference in the subsequent history. It would seem probable therefore either that one or more spermatozoa can enter the egg with no difference in the result, or, more probable, that there is some other mechanism to prevent the entrance of numerous spermatozoa. It is quite interesting to observe that at first the ovum has great attractive power for the spermatozoa, and all within a considerable distance are surely drawn in contact with it; but after the spermatozoon has effected its entrance, this attraction seems to diminish, and finally by the time that the segmentation begins, which is just after the fertilization proper takes place, the attraction has gone altogether, and the ovum, as far as concerns the active spermatozoon swimming around, is no more than a piece of inert matter. This peculiar power is certainly a highly remarkable one, for which no explanation can be given. As an experiment I mixed together the ova of Serpula and the spermatozoa of Thalassema. Serpula is an animal which agrees very closely with Thalassema in its development, but, as was of course expected, its eggs had not the slightest effect on the spermatozoa of Thalassema, while any Serpula spermatozoa which might chance to be present were seized upon with the greatest avidity. It is certainly calculated to excite great interest and amazement to think of the thousands of kinds of spermatozoa and eggs floating around in our waters, each one surely searching out its own proper mate and no other.

After the germinal vesicle has disappeared and the vitelline membrane has become distinct, there follows a rest of about fifteen minutes' duration. During this period nothing can be seen in the egg except occasionally the nucleus, which appears to change its position from time to time and usually approaches the surface of the egg. The first indication of any active changes is a quite remarkable swelling of the vitelline membrane at one point, which soon proves to be the position of the polar globules. The appearance of the egg at this time is given in Fig. 2. It would seem almost as if the vitelline membrane were endowed with some vital properties of its own, for, as the figure shows, this great protuberance appears before any signs of the polar globule are seen. Moreover, if the space between the egg and the mem-

brane be filled with liquid, any pressure would be equally diffused in all directions, and could not be so concentrated on one spot as to produce this protuberance. Further, as shown in Fig. 5, the vitelline membrane seems to share in the periods of rest and activity which the egg goes through, Fig. 5 representing a resting stage. Finally the vitelline membrane becomes the cuticle of the larva. It is not impossible therefore that it may be more than a simple dead covering to the egg; otherwise it is impossible to explain the various changes of shape it goes through. At all events, these peculiar movements of the membrane, which is entirely separate from the egg proper, are very puzzling.

Almost immediately after the above movement of the vitelline membrane there appears directly beneath it (Fig. 3) a small mass of perfectly clear, transparent protoplasm, which protrudes itself more and more into the space. As it increases the above-mentioned protuberance becomes more and more prominent, and finally when the polar globule is pushed off as a separate globule, it appears as a very large bunch (Fig. 4) upon one side of the egg, which has in the meantime become flattened.

After the globule is fully formed the egg appears to enter into a resting stage. The elevation of the vitelline membrane flattens down, the polar globule itself becomes quite flat, and the large space between the membrane and the egg almost disappears, and the egg once more becomes nearly spherical (Fig. 5). Everything indicates that this period has a significance similar to that of the well-known resting stages of segmenting ova. It occurs between two marked periods of activity, and is itself of about ten minutes' duration, a time during which not the slightest movements can be seen. It is finally closed by a second activity, which is nearly a repetition of the first. Once more the vitelline membrane is elevated into a prominent protuberance, and a second polar cell exactly like the first makes its appearance, pushing the first one as it grows further from the egg into the clear space between it and the vitelline membrane. As soon as the second polar cell is formed the first one divides (Fig. 8). So far as I could discover, this division of the first polar globule is the invariable accompaniment of normally developing ova. Up to this time the first polar cell has remained on top of the second one, but now it loses this position and comes to lie beside it. All three of the

polar globules are now more or less flattened; the vitelline membrane once more approaches the egg, and a second resting period ensues, during which, as shown in Fig. 9, we have the egg proper separated by a narrow space from the vitelline membrane, and in the space the three flattened polar globules.

What is this clear space? I have endeavored to decide this question, but have come to no definite conclusion. At first I was inclined to think it filled with a clear liquid, perhaps sea-water, which was squeezed out of the egg.[1] But two facts seem to show that this cannot be the case. In the first place the polar globules, which sometimes become entirely free from the egg, do not float around in the space, as would be expected if it contained a liquid, and secondly the clear substance shows a certain amount of definite structure. If the egg be examined under a high power, there can be seen extending from the egg toward the membrane very fine radiating lines, as in Fig. 13. They extend all around the circumference of the egg, and seem to indicate that this clear space is filled by something besides liquid. But, on the other hand, I found that its contents, whatever it may be, do not stain with coloring reagents. I came to the conclusion that the most probable interpretation was to consider it as being of a jelly-like consistency.

No sooner has the second polar globule become complete than there appears at the center of the egg a very large aster. This aster is plainly seen in spite of the many yolk-spherules, and its rays extend nearly to the circumference of the egg. Its interpretation is not difficult, although its formation cannot be studied. It is the union of the male and female pronucleus; *i. e.* the real fertilization of the ovum. The spermatozoon has remained in the ovum without making its presence visible, waiting for the final maturation; and now, after the germinal vesicle has cast out its superfluous part as polar vesicles, it is ready for union with the male element, and the union is indicated by the prominent aster. The preliminary changes and fertilization are now complete: the second resting period now occurring (Fig. 9) is of somewhat longer duration than the first, and somewhat longer than any of the subsequent ones, lasting usually about twenty minutes.

[1] Hoffman. Zool. Anz. 1880.

The development of the embryo proper now begins. It is noticed that the egg has become somewhat elongated in the direction transverse to the axis passing through the pole cells. On each half is now seen a nucleus surrounded by radiating lines and at first connected by an amphiaster, and this is immediately followed by the first segmentation (Fig. 10). The segmentation that follows needs no description. In the segmentation of the normal Thalassema egg it is perfectly regular and complete (Figs. 10–12). The divisions are into 2-4-8-16 segments, after which it becomes impossible to make out any regularity. Up to the division into eight segments the cells lie in their regular position (Fig. 11), but now they move upon each other and no longer lie in definite rows (Fig. 12). This, coupled with the fact that the cells are all alike, renders further observations more difficult. Each segmentation is followed by a resting stage, in which the segments appear less distinct, and the egg appears almost as if unsegmented (Fig. 14). It is just possible in all cases to make out the boundaries of the cells very indistinctly, as Fig. 2 shows. A new segmentation is always inaugurated by a swelling of the segments, by which they become once more distinct, and this is immediately followed by division. Again appears a resting stage, and again a period of activity. Thalassema thus presents no anomaly in this respect, but simply adds one to the list of eggs which segment with these alternating periods of rest and activity—a list which bids fair to be found universal.

In a more general way there are a number of quite interesting points in connection with the development of the egg thus far. First, in regard to the relation of the polar cells to the entrance of the spermatozoon. As is indicated above, there is a direct connection between the two in the case of Thalassema. The ova remain in the mother in a mature condition, but entirely dormant. They may be put into sea-water and left there until they go to pieces, but still no indication of any life in them. But as soon as the first spermatozoon enters the egg they are immediately roused from their dormant condition and begin to go through rapid changes, which result in the throwing off of the polar globule. There can be no doubt then that the protrusion of the polar globules is in some way influenced by the entrance of the spermatozoon.

It is pretty generally acknowledged now-a-days that the polar globules are thrown off entirely independent of fertilization, since the impregnation may take place either before or after or during the process of their formation.[1] Balfour even goes so far as to say "there is no evidence to show that the process is influenced by the contact of the male element." In his Elasmobranch Fishes he says that in order to show that the two processes are in any way connected it will be necessary to bring forward instances where the polar globules are not thrown off unless the egg is fertilized. One or two such instances have now been found. It is true of two species of oyster.[2] It appears also, according to Hoffman, to sometimes be the case in certain bony fishes (*loc. cit.*) In the present case we have another well-marked instance of the same, in which the polar globules are never thrown off before the spermatozoon enters the egg. It is therefore incorrect to say that the process is always uninfluenced by the male element.[3]

But even in these cases there is no reason for thinking that the polar cells have anything to do with fertilization. Fertilization does not occur for some considerable time after the entrance of the spermatozoa; not until after the polar cells are entirely extruded, as we have seen. What really occurs is this. The ovum is, when put in the water, in a completely dormant condition; perfectly capable of maturing itself if the appropriate stimulus be supplied, but not capable of itself to go through any changes. It may be roughly compared to a muscle which unless stimulated will die without any contraction, but has nevertheless the power for considerable work. In the egg this stimulus is supplied by the entrance of the spermatozoon, which rouses the egg from its inactivity. The changes which take place, however, have really nothing to do with the spermatozoon, which simply remains in the egg, without uniting with it, until all the preliminary changes have passed. Then occurs the proper fertilization, and from this time the activity is the result of both male and female elements. The extrusion of the polar cells is therefore just as truly the maturation of the egg as in those cases where it occurs entirely independent of the male element.

[1] Hertwig. Morphologisches Jahrbuch, I–IV.

[2] Ryder. Emb. of Osseous Fishes, 1884.

[3] See Hyatt on Cellular Tissues. Proc. of Boston Soc. XXIII.

It has for a long time been understood, pointed out first by Hertwig,[1] that the protrusion of the polar globules is a process in certain respects homologous with segmentation, that the polar globules have the value of cells and arise by a process of cell-division. The internal changes accompanying their formation are almost the same as those of segmentation; the amphiasters accompanying division of nuclei are seen and each pole-cell is supplied with its own nucleus. The two processes differ chiefly in the fact that one of the resulting segments is smaller than the other. Moreover, as is shown in the present instance, the polar cells are capable of still further development; slight, of course, but still positive. In Thalassema, one of the cells divides into two. In certain insects, a body which is considered by Weismann[2] to be a polar globule is capable of still further division, giving rise to as many as a dozen segments.[3] Everything indicates that the polar globule is a cell. This conclusion is of some considerable importance when we come to consider the significance of these bodies, and seems to tell quite strongly against that class of theories which considers them as simple excretions of the egg. It seems also to me to be an argument against the view of Balfour and Minot which finds in the polar globules the male portion of the egg cell.

In Thalassema I have found another peculiarity which, taken in connection with the reasons already given, may be considered to form an argument for considering the protrusion of the polar globules and segmentation as homologous processes. As mentioned above, segmenting eggs present almost universally rhythmically alternating periods of activity and rest; a fact which, in consideration of its wide occurrence, must have considerable meaning in egg physiology. From the description given above it will be seen that Thalassema presents precisely similar alternating periods in its process of polar globule formation. The first period of activity is followed by a marked period of rest, this again by a second period of activity and a second rest. In every respect do these resting periods seem similar to those of the

[1] *Loc. cit.*

[2] Weismann. Beiträge zur Kenntniss der ersten Entwicklungs-Vorgänge in Insecten, VI.

[3] Whether these bodies be polar cells is at least doubtful, and their division may be nothing more than degeneration.

segmenting egg. The polar cells flatten down and become indistinct precisely as do the segments of the egg, and each new active period is inaugurated by these swelling out once more in distinctness.

Having noticed this rhythm so marked in every instance, it occurred to me to inquire if by careful study it could be any more closely compared with that of the segmenting ovum. For this purpose I studied closely a large number of eggs, noting carefully the times of the various changes, following in each case a single egg through its history till the time of 16 segments. The resting periods in the table below are as follows: 1st. Between the protrusion of the first polar globule and second. 2d. Between second polar globule and the first segmentation. 3d. Between the first segmentation and the second, and so on. The table gives the result of only a few of the observations upon this point; many others were made, but they agreed almost exactly with these.

	Egg 1.	2.	3.	4.
Duration of 1st resting stage,	10 min.	10	11	10
" " 2d " "	15	18	22	18
" " 3d " "	18	20	18	17
" " 4th " "	18	16	...	15
" " 5th " "	17	19	17	17

I have not thought it necessary here to give the duration of the active periods, since they are all so brief and so near alike as to make it next to impossible to distinguish them. It will be seen that the duration of the first resting stage is quite different from that of the others. Each period varies to a considerable extent in different eggs, and the different periods vary also in the same egg; but the resting periods of the segmenting egg do not differ among themselves as much as they differ from the first resting stage. This was in every case either 10 or 11 minutes, while the 3d, 4th and 5th, etc., varied only slightly either side of 18 minutes. The second period—viz. that between the protrusion of the polar cells and the segmentation during which we have seen the true fertilization of the egg takes place—has a wider variation than any of the rest, varying from 15 to 22 minutes. This table then teaches us that the rhythm of rest and activity

does not begin with the segmentation, but makes its appearance with the earliest activity of the egg, although there is something of a break just before segmentation begins; and we are justified in drawing the conclusion that the physiological processes governing the protrusion of the polar cells is in part the same as that governing segmentation. In itself, of course, this is not enough to indicate that it is a process of cell-division, or in any sense homologous with segmentation; but taken with the other evidence which we have, it is certainly an additional argument. In saying that it is homologous with segmentation, it is, of course, not meant that it is the beginning of the formation of the embryo, but simply that it is just as truly a process of cell-division, and not to be considered as a process of excretion.

It may be worth while finally to call attention to the fact that the polar cells are extruded into the space between the egg and the vitelline membrane, and not, as is frequently the case, extruded through that membrane.

It is an interesting and somewhat surprising fact to discover that the segmentation of the ovum in Thalassema is a perfectly regular and total segmentation. Little doubt remains that the Echiuridae are highly modified annelids. The development of Echiurus as given by Hatschek[1] is certainly the development of an annelid larva; the earlier stages, moreover, as will appear in this paper, agree so closely with that of the primitive annelid Serpula as to leave scarcely room to doubt that they are modified annelids. Now, as is well known, all annelids which have been described, with possibly two exceptions, have a segmentation which is more or less irregular—usually, in fact, very irregular. The two exceptions are Serpula[2] and Terebellides.[3] That Thalassema and Serpula should have, contrary to the rule, a regular segmentation is a fact that requires a little consideration, and, if possible, an explanation.

It is well established that the presence of a large amount of food-yolk usually leads to an irregular and incomplete segmentation. The food collects at one pole of the egg, and mechani-

[1] Hatschek. Ueber Entwick. von Echiurus. Arb. a. d. Zool. Inst. Wien, III, 1880.

[2] Stossich. Beiträge zur Ent. d. Chaetopoden. Sitz. d. K. K. Akad. Wiss. Wien, B. LXXVI, 1878. This fact I have also myself confirmed.

[3] Willemoes Suhm. Zeit. f. wiss. Zool. XXI, 1871.

cally interferes with the divisions which concern the protoplasm only; the protoplasm is at the same time mostly collected at the opposite pole, and as a result this pole segments rapidly, while the pole with the food-yolk segments only very slowly. But all this is not necessary, even in the presence of considerable food-yolk. In the present instance we have an egg with a large amount of food-yolk, nearly, if not quite, as much as is found in many annelids with irregular segmentation; but there is a difference in this point, that in Thalassema the food-yolk is uniformly distributed through the egg, and is not accumulated at one pole. Uniformly distributed food-yolk, even though it be abundant, cannot interfere with the segmentation. I would now suggest that a partial explanation for this peculiarity of Thalassema may be found in the circumstances under which the egg goes through its early development. Its eggs are of nearly the same specific gravity as the sea-water, and it is in this medium that they undergo all of their development. When ripe they are cast into the water, are there fertilized, and there do they develop, floating freely all the while. Now we certainly should expect that under these circumstances the relation of the food-yolk would be different from those cases where the egg is comparatively stationary. Evidently, if an egg is placed in a medium of about its own specific gravity, and if it be floating freely in this medium, the force of gravitation will be almost entirely absent, and there would be nothing to cause the food-yolk, if it be present, to collect at either pole of the egg; gravity would act in no particular direction, and the tendency would be for the yolk to become uniformly distributed through the egg, and there would consequently result a regular segmentation. On the other hand, when eggs are deposited in special stationary capsules, where they pass their early development in the body of the mother, or where they are placed in any way under circumstances where gravity can have its full influence, we should expect that the food-yolk, being either lighter or heavier than the protoplasm, would collect at one pole and thus lead to an irregular segmentation. The rule among annelids is that the eggs are laid in stationary capsules, and here gravity exerting its influence, has induced an irregular segmentation. This suggestion, which occurred to me some three years ago, has

lately received some interesting confirmation from the experiments of Pflüger.[1] The results of these experiments were in brief to show that the direction of the action of gravity has very great influence upon the segmentation of the egg of the frog, the character of the segmentation being entirely changed when the eggs were so turned that the ordinary perpendicular axis of the egg was inclined. The publication of these experiments convinced me more strongly than ever that there is some connection between the condition under which the early development of eggs is passed, and their kind of segmentation.

Does embryology give us any reasons for thinking that this really represents a fact? If it were true, we should expect to find freely-floating eggs with a regular segmentation, and eggs in any way rendered stationary, or protected in their early development, with an irregular segmentation; but this is far too general to give much hope of finding it true. If it be admitted that a regular segmentation is more primitive and preceded an irregular one, we should with reason expect to find that eggs which float freely in the water had retained this regular segmentation, not having had the opportunity to acquire an irregular one. We might not expect that in every case where the egg had become stationary there would be an accumulation of food-yolk enough to produce an irregular segmentation; but we should expect that those eggs which have acquired an irregular segmentation would belong to the class of protected ova, or be descended from such a class. And, further, we must not be surprised if some seeming exceptions occur.

If now, bearing these points in mind, we take a brief survey of the segmentation in various animals, we shall find that the facts are quite in accordance with the view. In the first place, animals which lay their eggs in protected positions nearly always have some irregularity in their segmentation. Annelids, as already mentioned, have an egg which passes through a markedly irregular segmentation, leading to the formation of an epibolic gastrula; at the same time they almost always agree in depositing their ova in special capsules, or in carrying them about during the early stages of development, or in some way protecting them. Serpula, Terebellides, Thalassema and Sabel-

[1] Pflüger's Archive, 1883.

laria are the only exceptions to the irregular segmentation of which I am at present aware, and it is certainly a very interesting fact to find that Serpula, Thalassema and Sabellaria cast their ova into the water for development; and to make this fact of still more importance, it is known that in the case of Serpula and Thalassema (the other forms have not yet been studied) there is the best of evidence that the entire development preserves very closely the primitive type. In the annelids then the primitive history is that exemplified by Serpula, which has a regular segmentation and an invaginate gastrula. In instances where the eggs are still cast into the water, and where the larva must take care of itself, this primitive form has been retained. In most cases, however, the annelids have acquired the habit of protecting their eggs in some way; the eggs, becoming stationary, are acted on by gravity, and the food-yolk under its influence has aggregated at one pole of the egg, giving rise to the irregular segmentation.

Polyzoa, Hirudinea, Tracheata, Crustacea are groups of animals which as a rule possess an irregular segmentation, and in none of these cases, with a very few exceptions, do we find freely floating ova. The Platyelminthes are not well enough understood to give very valuable evidence, but it may be pointed out that Turbellarians and Trematodes have an irregular segmentation and a protected egg, while Nemertians have a regular segmentation and a freely floating egg. The vertebrates also come under the rule. In the one vertebrate which all observers agree has the most primitive history (Amphioxus), the segmentation is regular and the eggs are ejected into the water. In other vertebrates it is very rare to find a floating ovum, and it hardly need be said that it is also rare to find a regular segmentation. Some bony fishes cast their ova into the water and at the same time have an incomplete segmentation; but this is so unquestionably a very recent modification that the exception is of no weight. In Platyelminthes, Polyzoa, Annelids, Discophora, Tracheata, Crustacea and Vertebrates (for mollusks see below) we have instances where an irregular segmentation is accompanied by a protected ovum.

On the other hand we find the large group of Echinoderms which eject their ova into the water for development and have

(with one single exception, Echinaster Sarsii) a regular and total segmentation. The Chaetognaths have the same. The Coelenterata, if we leave out the Ctenophora—which seem to be exceptions, although their early development is still very incompletely understood—conform quite well to the rule. It is certainly the rule here that a regular segmentation is accompanied by a free ovum. This is true of Actinozoa, Alcyonaria and many Hydrozoa. Some Hydrozoa have their ova in stationary gonaphores and still retain an approximately regular segmentation, though very frequently at least not strictly so, as may be easily seen by study of living specimens. Indeed, so great is this irregularity in Tubularia that Ciamucian[1] described it as an epibolic gastrula, and, although later results[2] have shown this to be an error, yet the irregularity is evident enough. On the whole, then, the Coelenterata, although they present greater difficulties than other groups, conform quite well to the rule.

The most interesting examples are presented by the Mollusca, for we here get some evidence that not only may eggs acquire an irregular segmentation by becoming stationary, but the subsequent assumption of free ova may gradually eliminate this. At first sight mollusks seem to present difficulties, but they are difficulties which in reality give us the most positive evidence. As a rule mollusks deposit their ova in cocoons, in jelly-like masses, under stones, or in some way protected, and partially stationary, and with this habit, as we would expect, is found an irregular segmentation. But among the Lamellibranchs are found instances where the ova are freely floating, and at the same time we find an irregular segmentation. The best understood instance of this kind is the American oyster. Now, this is a case where the habit of ejecting the ova into the water is a lately acquired one. Modern investigations[3] indicate that the Gasteropoda are the more primitive forms, while Lamellibranchs are much more highly modified. The Gasteropods never have a free ovum, and, moreover, most Lamellibranchs retain their eggs in their gills. Even the European oyster differs from the American oyster in this respect. This habit of the oyster, then,

[1] Ciamucian. Zeit. f. Wiss. Zool., XXXII.
[2] Hermann. Jenaisches Zeit., XV.
[3] See Brooks and Lankester.

must be a lately acquired one. It is quite interesting, consequently, to inquire if it has had any effect on the segmentation. An examination of the work done by Brooks[1] shows that it has. Without repeating his arguments, it is sufficient to say that he shows that the rather peculiar segmentation of the oyster has been brought about by a loss of food-yolk, which has caused the segmentation to approximate to a regular one, while still retaining by heredity the traces of the typical molluscan irregularities. Putting this conclusion with the lately acquired free ova, and we certainly have a pretty piece of evidence that the type of segmentation and the method of depositing the ova are connected. The peculiarities of molluscan segmentation may perhaps be partially explained in this way. Gasteropods in general have acquired the habit of depositing their ova in egg cases, etc., and with this habit a large mass of food-yolk and an irregular segmentation. Lamellibranchs in general protect their ova in a different manner by carrying them in their gills, and this may explain the fact that their segmentation differs to a considerable extent from that of the Gasteropods, as shown by Rabl.[2] The oyster with its newly acquired free ovum is still undergoing a loss of food-yolk. In *Unio* there has been a migration from the sea to fresh water, and this always having great effect on the embryology, may account for the peculiar segmentation.

This view is of course little more than a suggestion. If it be correct, we should not look for sudden changes; and should some animal suddenly acquire a new method of depositing its ova it would be a long time before we could expect its segmentation to be modified, and we should therefore expect to find some exceptions to our rule; but the presence of great groups, such as Echinoderms, Annelids and Mollusks, which do so closely coincide with this view, seems to me to be sufficient reason for accepting it. The object of food-yolk, as is well known, is to enable the young to abbreviate its development by having its food supplied to it, and being consequently able to skip some of its ancestral stages. That this should take place, it is

[1] Brooks. Development of Oyster and Loss of Food-Yolk in Molluscan Eggs. Stud. from J. H. U. Biol. Lab. I.

[2] Rabl. Entwick. der Tellerschnecke. Morph. Jahr. V.

necessary not only that the young should be supplied with food, but also that it should be protected during its early history; and if now it be admitted that the habit of depositing the egg has a direct effect on the acquisition and loss of food-yolk, we have an interesting addition to our evidence as to the effect of environment on embryology, and have a mechanical explanation for many of the peculiarities of segmentation.

III.—*Gastrula.*

By this regular segmentation the ovum becomes first a solid mass of cells, a morula (Fig. 12), and later a blastosphere (Fig. 15). The formation of the blastosphere occurs very early, by the formation of a segmentation cavity. By the time the segmentation has reached 32 segments, it can be seen by section that they no longer lie closely together, but have somewhat separated so as to leave a cavity at the centre of the egg. Fig. 15 is an optical section, showing the segmentation cavity at this time of its first appearance. The cavity grows somewhat larger as segmentation proceeds, but never becomes very large.

Meantime, cilia have made their appearance, first being seen at about the same time as the segmentation cavity. They arise in great numbers, and protrude through the vitelline membrane (Fig. 15). The vitelline membrane is thus transformed into a cuticular covering for the young larva, and this covering is retained throughout the larval life. There is therefore nothing which can be considered to be the hatching of the embryo, the growth being a continual one, in this agreeing with Serpula, in which the vitelline membrane also remains as the larval cuticle. The cilia when they first appear seem to be uniformly distributed, but a careful study shows that this is not true. They are really confined to a broad band extending around the egg, leaving the two poles of the egg free. At one of these poles are the cells which are soon to become the endoderm cells. They are not in any way distinguishable from the rest of the cells, with the exception that they have no cilia. At the other pole are cells which are to become the larval nervous system, and which soon develop cilia of their own.

As soon as the cilia appear the embryo begins to move. The

motion is, however, very indefinite, being in no particular direction, and with no one surface in advance, but simply a more or less regular revolutionary motion. There is as yet nothing to distinguish anterior extremity from posterior extremity, the one broad band of cilia being the only feature of definite location. By the motion now acquired, and also by a changed specific gravity in part, the embryos now rise to the surface of the water, and are now to be found collected in great numbers around the edges of the aquarium where they are confined. This collecting in crowds, however, does not last very long, and simply indicates that the larvae have no control over their motions. As they grow a little older and an anterior region becomes distinguished from the rest of the body, their revolutionary motion becomes changed into a partially direct one. Gradually they seem to get control over their motions, and are finally able to move in a definite direction, with their head-end in advance. After this they no longer collect in crowds, but swim at will through the water, perhaps at the surface, perhaps below it. This is not accomplished, however, until the larva is about three days old. The mechanism by which the change is effected we shall see later.

Very soon after the cilia have arisen, the embryo is transformed into a gastrula by a modified form of invagination. The cells at one of the poles of the egg, free from cilia, become transformed into an endodermal sac by a process that is not a typical invagination such as occurs, for instance, in echinoderms, but is partly an infolding and partly an ingrowth of cells. On external view this cannot be understood, but a section (Fig. 16) shows the real condition. There is a slight infolding of the cells made evident by a flattening of the egg, but the chief change is an ingrowth of each endodermal cell. The endodermal mass thus formed is not a hollow sac, but a solid mass of quite large cells, with no distinct blastopore. The external ends of the cells are connected with the ectoderm at a region which is homologous with the blastopore, *bl.*

A second important organ has also become visible. If the ectoderm immediately opposite the blastopore be closely examined, it will be seen that the cells here are larger than the other ectodermal cells, a quite noticeable thickening being evident at this point. From these cells arise a number of cilia

which are very different in character from the others in the animal. They are very long, and are not movable, but are carried protruding stiffly, as shown in Figs. 16–23, etc., in marked distinction to the other cilia of the band, which are short and are rapidly moving. The future history of this ectodermal thickening proves it to be the beginning of the nervous system of the larva (the *schedelplatte* of the German writers), and the cilia are undoubtedly sense-cilia. Such a tuft of cilia is of almost universal occurrence among larval forms, and is usually, as here, the first indication of any region of special sensibility, and consequently of the nervous system of the larva. It may consist of a tuft of long cilia, as in the present instance; it may consist of a few exceedingly long cilia, as in Actinia,[1] or lastly, it may consist of only one long, stiff hair, as in the case of the pilidia of Nemertians.[2] In all cases it is directed forward in swimming, and it always appears early in the larval history. In the present instance, even before the mouth is formed, it indicates that even thus early has the larva become distinguished into an anterior and posterior part.

From this time on we can point out definite regions in the larva. The end with the sense-cilia is anterior, and even now can we see that it begins to be functionally as well as morphologically the anterior end, since it begins to be carried in advance as the animal swims. The other extremity of the egg carries the blastopore, and we would naturally call it the posterior end; but the development shows us that this is not true. For the present we will therefore simply call it blastopore extremity. Between the two extremities is the ciliated band above mentioned (Fig. 16). This ciliated band has by this time somewhat changed its character. The band has become narrower, and the cilia have become rather longer and more powerful. This band, becoming more constricted in breadth, gives the band greater power of motion. If the larva were entirely covered with cilia it would be hardly possible that there should be any motion other than an irregular revolution; but the cilia, being confined to one band, are able to produce a certain amount of onward motion, and as this band becomes narrower and the cilia become

[1] Lacaze-Duthiers. Arch. d. Zool. Exp. et Gen., Vols. I and II.
[2] Metschnikoff. Mem. Acad. St. Petersburg, Ser. VII, t. XIV, No. 8.

stronger, the power of controlling their motion becomes greater and greater.

The digestive tract, as above described, is at first a solid sac of large cells. Very soon, however, there appears in it a cavity which opens to the exterior at the blastopore. But other changes occur at the same time which lead us to the fourth division of the subject.

IV.—*Formation of the Trochosphere Larva.*

The embryo has now, at about the eighth hour, reached a form represented in Fig. 16, a gastrula with the blastopore at one pole, with a circumoral band of cilia at a short distance from the blastopore, and with a tuft of sense-cilia at the opposite pole, the anterior extremity. As yet the digestive sac is solid, and the only indication of the blastopore is a slight flattening of the egg. This flattening, and therefore the blastopore, is at first directly opposite the sensory tuft of cilia and in the centre of the circumoral ring. The point is of importance in enabling us to understand the meaning of the resulting trochosphere larva and its relation to other larval forms, and needs to be clearly understood. From this point the digestive tract extends into the segmentation cavity, nearly filling it, and at first ending blindly. The embryo swims as yet in an irregular manner, but for the most part with its sense-cilia in advance.

Two changes now take place almost simultaneously. The first is the appearance of a cavity in the digestive tract. This cavity would seem to arise by an absorption of the central parts of the digestive mass, not at first communicating with the exterior. Only a short time, however, does this state of affairs last, for an opening to the exterior soon appears which we may as well at once call the mouth. This opening does not appear directly in the centre of the flattening which we have considered the blastopore, but slightly to one side, so that it is nearer the circumoral band upon one side than upon the other (Figs. 17, 18). This gives us immediately a means of distinguishing the ventral and dorsal surfaces, for the mouth of the larva is ventral in position.

We have now distinguished in our embryo two regions, an anterior, with the sensory cilia, and a ventral, with the mouth,

but as yet we cannot say where is the posterior extremity. For, indeed, the posterior extremity is not yet formed.

The second change which has occurred is the division of the digestive tract into two distinct regions. There occurs at a short distance from the mouth a constriction (Fig. 17) separating the digestive tract into a stomach and an oesophagus. This oesophagus becomes almost immediately lined with cilia, and from this time remains as a distinct region opening at its interior into the stomach and at the exterior opening by the mouth. The blastopore is therefore converted into the mouth. This is perhaps not strictly true, since, owing to the peculiar method of the invagination, no blastopore is formed until the cavity in the digestive tract communicates with the exterior as the definite mouth, but essentially it is correct, for the mouth appears in the exact region of the blastopore as the first opening. At all events, the blastopore does not become the anus, which is not formed till some time later.

Having once become differentiated, the stomach and oesophagus become more and more unlike each other. The oesophagus rapidly elongates, and becomes converted into a cylindrical tube of considerable length, whose walls are composed of columnar epithelial cells richly ciliated on the inner surface (Fig. 19). The stomach for a while only elongates very slightly, still retaining its spherical shape, and almost filling the segmentation cavity. The method by which the radically symmetrical gastrula of Fig. 16 becomes converted into a complex, rapidly moving, *bilaterally* symmetrical trochosphere larva is very remarkable and interesting. The development of bilateral from radial is naturally considered as brought about by growth, by the elongation of the body. But this elongation may be looked upon as taking place in different directions, and the significance which we place upon the different regions of the body in bilateralia will depend largely or entirely upon our way of looking at this change. In the present instance we have an excellent opportunity to study this growth in the ontogenetic series, and I have therefore spent considerable time in making out the exact method of the change. The transformations are somewhat difficult to understand. At the time of the invagination the embryo swims with the blastopore almost directly opposite the sense-cilia, and, as these are

undoubtedly anterior, we might think that the blastopore was posterior. But we have seen that the blastopore becomes the mouth, and a glance at the completed trochosphere larva (Fig. 28) shows us the mouth much nearer the anterior end than the posterior end. And if we compare this with the adult (Fig. 43), or, better still, examine the adult of an ordinary annelid such as Polygordius,[1] it will be seen that the mouth comes to be at almost the anterior end. The reason for this is that the elongation of the body concerns entirely one side of the post-ciliated region. Fig. 16 is a representation of the embryo shortly after invagination. The sensory cilia are at the anterior extremity, and the blastopore is nearly opposite. The body is slightly flattened. Fig. 18 is the same a short time afterward. The digestive tract has differentiated into oesophagus and stomach, and the oesophagus has somewhat elongated itself. The mouth has slightly changed its position and now lies quite near the circumoral ring. Now the body of the larva begins to elongate, and the elongation is not in a direction to carry the mouth backwards. The line *AB* indicates the direction of growth, and it will be seen from Fig. 18 that the growth in length is confined to the region lying between the mouth and the opposite side of the ciliated ring, and is therefore entirely post-oral. Accompanying this elongation, the digestive tract has also grown, and has of itself become longer than the body-cavity. It is necessary, therefore, that it be somewhat doubled upon itself, and since the elongation principally concerns the oesophagus, it is seen that the stomach has become folded upon itself so that its long diameter is nearly parallel to that of the oesophagus and its free end projects into the elongated part of the body (Fig. 19). Its position now is almost exactly the reverse of its original position (Fig. 16). The elongation still continues, and finally the anus is formed at *a* (Fig. 19). This region is now, of course, the posterior extremity of the body. Now, if we compare Fig. 16 with Fig. 19, two important points are evident. The embryos seem at first to have been reversed. In Fig. 16 the mouth (blastopore) is almost exactly posterior, while in Fig. 19 it has moved forward to be near the anterior extremity, but still posterior to the ciliated band. And secondly, if we look in Fig.

[1] Hatschek. Arb. a. d. Universitaet, Wien, III.

16 for the point where the anus is to appear, we find it at *a*, very near the mouth, and, indeed, within the original flattening of the egg which we have seen to be the blastopore. Its present position at a considerable distance from the mouth is due to the elongation of the larva in the direction indicated by the line *AB*. It is not correct, therefore, to say that the anus is formed from the blastopore, but it is formed at a position which corresponds to one end of the elongated blastopore. The extreme significance of this peculiar method of formation of the trochosphere I have shown in a previous article on larval forms in these studies.

The alimentary canal is completed in about twelve hours, the intestine and anus appearing as follows: As the body grows, the stomach also grows and continually fills the body-cavity. This cavity is nothing but the segmentation cavity, which has become very much increased in size. Thus in ten hours an embryo such as is seen in Fig. 19 is formed. Now, as the whole animal continues to grow, the posterior part of the alimentary canal, which I have hitherto called the stomach, becomes divided into two portions. If the embryo be viewed from the dorsal surface, two partitions may be seen growing inward near the posterior from the walls of the digestive tract. These partitions, in reality simple constrictions, thus have separated a stomach proper (Fig. 20, *st.*) from a smaller intestine, *i.*

Thus far the whole alimentary tract lies freely in the body-cavity, being united with the body-wall only at the mouth, the other extremity ending blindly, the relations being just as in the gastrula, except that it has become longer and more complicated. But as soon as the intestine becomes differentiated its free extremity unites with the body-wall at the posterior end of the body, its walls become fused with the ectoderm, and for a short time the two form a wall of double thickness. This soon breaks through, however, and thus a posterior opening is formed to the alimentary canal. The canal is now complete, and consists (Fig. 27) of the usual divisions—oesophagus, stomach and intestine—and is from this time functional. It will be noticed that neither the oesophagus nor the rectum is formed as a distinct invagination—*i. e.*, there is neither true stomodaeum nor proctodaeum, and on this point Thalassema differs from the ordinary rule. It

has been shown, however, by Hatschek[1] that in the mollusks the intestine is really an endodermal formation, and not formed as an ectodermal invagination. In this article he suggests the possibility (p. 35) that future investigations might show the same to be true of annelids, contrary to what he himself had concluded from the study of Polygordius. We now see that this suggestion of his was justified, for the intestine is undoubtedly in this case of endodermal origin.

V.—*The Trochosphere Larva.*

By the changes above described the ovum has become converted into a type of larva known as the trochosphere, which is complete about twenty-four hours after fertilization. The larva is small (at first scarcely larger than the egg), partially transparent, and swims through the water at all depths with a perfectly regular motion. Fig. 27 is an optical section through such a larva, and Fig. 28 is a tolerably good representation of a surface view. It consists of a regular oval body, noticeably divided into two parts, a pre-oral lobe and a post-oral lobe, separated from each other by a ciliated band, at first near the middle line of the body. The relative size of the pre-oral and post-oral lobes varies somewhat in different individuals, the pre-oral lobe being at first, commonly, slightly longer, but very soon being surpassed by the post-oral lobe. Just posterior to the ciliated band is the mouth (Fig. 27, *m.*), which opens into the digestive tract, consisting of oesophagus, stomach and intestine, with an anus at the extreme posterior extremity of the body.

The ectoderm does not present everywhere the same characteristics, but has already developed various ectodermal organs. We may, for description, divide the ectoderm into four different regions: 1. The ciliated band separating the pre-oral and aboral lobes. 2. The beginning of the supra-oesophageal ganglion. 3. A ventral band of cilia extending from mouth to anus.

1. The distinctive characteristic of a trochosphere larva is the presence of a pre-oral ciliated band (Fig. 27, *pr.*). We have already seen that the gastrula has a circumoral band of cilia, and that this becomes converted into the pre-oral band of the

[1] Ueber Entwicklungsgeschichte von Teredo, Arb. a. d. Inst. Wien, III.

larva; but upon examining a full-grown trochosphere it is seen that the pre-oral band we find here is not identical with that of the earlier band. We have hitherto seen a broad band of cilia arising from cells which form a prominent ridge around the larva in front of the mouth (Fig. 19). Such a broad band is present from the first appearance of cilia until about the middle of the second day, when it is replaced by the proper ciliated ring characteristic of the complete trochosphere. This consists of a single row of very long, powerful cilia (Fig. 27), which form a much more effectual locomotor organ than the ciliated band of the younger stages, owing, no doubt, partly to the greater power of the cilia and partly to the more definite regularity of their motion. That this is so, is evidenced by the greater power of motion which the larvae from this time possess. By study of stained specimens it can be made out that these cilia arise from a very definite row of large cells. From a surface view (Fig. 36), or from optical section, it is seen that these cells are very different from the surrounding ectoderm, being high, prismatic cells with distinct nuclei, each cell being the origin of a large number of cilia. Moreover, staining fluids readily differentiate them, which is not true of the rest of the ectoderm.

Posterior to the mouth is a second row of cilia of precisely the same appearance as the pre-oral row, and arising from a similar band of prismatic cells (Fig. 36). Upon the dorsal surface these rows are quite close together, but ventrally the post-oral band is somewhat curved, so as to enclose between it and the pre-oral band the mouth (Fig. 28). The cilia of the post-oral row are not so powerful as those of the anterior one, and probably serve largely for collecting food, as well as assisting in locomotion.

The pre-oral band of cilia is thus composed of two different sets of cilia. At first it consists of a broad band of small cilia, which are so widely dispersed that they can give no regular motion to the animal. This band soon disappears, and is replaced by the single row of long cilia. Whether this row be a newly-formed one, or whether it be a row which was present in the original broad band and has become more developed with the disappearance of the rest of the cilia, does not alter the fact that

it is a distinct formation. These two different sets of cilia have considerable significance towards an understanding of the meaning of a pre-oral ciliated band.[1] It may be well to point out their different meanings in the life of the larva. The broad band, as we have seen, gives an irregular motion, which lasts about two days. The object of this motion must be to distribute the larvae as widely as possible, and it accomplishes this partially by the intrinsic motion of the larva, but more effectually by bringing the animal to the surface of the water, where it is more readily acted upon by the various surface-currents and the winds. Undoubtedly, it is of great advantage to the species to have its larvae dispersed as widely as possible while it is yet feeding upon the food in the yolk and before it is required to capture food for itself. After the mouth and anus are formed, and it becomes necessary that food from without should be obtained, a more direct motion is desirable, particularly a motion which will enable the larva to swim beneath the surface of the water, since its food consists of microscopic plants which are not readily found at the surface. For this purpose the broad band becomes more and more constricted, and finally is confined to a single row of cilia (which are more likely developed anew), much more powerful than the old band, and calculated to give the larva a very definite motion, well calculated for its own advantage, but not so well adapted for the distribution of the species as the irregular motion of the first day.

2. The supra-oesophageal ganglion has already made its appearance at a very early stage (Fig. 16), and is now seen as a prominent thickening of the ectoderm at the anterior extremity (Fig. 27, *sp.*). This thickened plate is made up of large cells, whose outlines are more distinct than in the rest of the ectoderm, and can readily be differentiated in stained specimens. Figs. 22, 23, 31 show this thickening in various stages. It is always crowned with the tuft of sense-cilia.

3. Besides the pre-oral and post-oral ciliated rings, we find a broad ciliated band occupying the ventral median line (Fig. 27, *vc.*). This band of cilia is connected with the ventral nervous chain in the same manner as is the tuft of sensory cilia with the supra-oesophageal ganglion; the cells from which the cilia arise

[1] See a paper on larval forms in the previous volume of these Studies.

are later to give rise to the ventral nerve-chain. This band also appears very early in the development, being present some time before the anus is formed, and for a long time—a number of weeks—it is the sole representative of the ventral portion of the nervous system, the nerve-chord not being differentiated until quite late in larval life. The ectodermal cells within this band differ very much from the ordinary ectodermal cells. Not only are cilia present, but all of the peculiar structures (glands, pigment-bodies, etc.) found in the ectoderm elsewhere are absent from these cells. There can be no doubt that this region is especially differentiated. The presence of such a ventral ciliated band is quite as common an occurrence among larvae, and seems to be the antecedent of the ventral nerve-chord. Even in Lumbricus,[1] where the free larval life is lost on account of the presence of food-yolk, this ciliated band is retained, although it cannot be of any use to the animal, as far as we can see. If Kleinenberg's figures be compared with Fig. 41, it will be seen that in Lumbricus and Thalassema the relation of this ciliated band to the developing nervous system is essentially the same. We find, thus, that both the cerebral ganglion and the ventral nerve-chord are preceded by cilia, and the intimate connection of cilia and nerve-cells in these cases suggests that possibly the pre-oral band of cilia may have some such signification.

In front this ventral band is prolonged into the space existing between the ab-oral and pre-oral ciliated rings, which is also richly ciliated (Fig. 28). The cilia are much shorter than those of the pre-oral ring, as (Fig. 29) a section through the body-wall near the mouth will show. Fig. 32 is a similar section through the dorsal region, where the pre-oral and ab-oral rings are nearer together.

There are some other cilia scattered quite irregularly over the body.

The appearance of the ectoderm outside of the three ciliated tracts above mentioned is represented in Fig. 21. I have found it impossible, with any histological method at my command, to differentiate the cells and make out exactly the histological structure. There are a number of bodies, however, lying in the

[1] Kleinenberg. Development of the Earthworm Lumbricus trapezoides. Q. J. M. S. XIX, 1879.

ectoderm. First, we find the ectoderm, at all times after the first day, crowded with peculiar unicellular glands (Fig. 21, *gl.*). These are worm-like bodies, very highly granular, and occur in great numbers all over the body of the animal (Fig. 28). They produce an abundant slimy secretion, which makes it difficult to treat the larvae with any hardening reagents. As soon as any acid comes in contact with the body these glands discharge their contents, causing the larvae to become very sticky and to adhere firmly to the bottom of the vessel in which they happen to be. The discharge of the glands causes the ectoderm to swell to great size and prevents staining fluids from readily penetrating to the interior. Fig. 31 is an optical section through the ectoderm showing the glands, and Fig. 30 is a section of the same after treatment with osmic acid. Nothing is to be seen of the glands except the nuclei.

From the second day the ectoderm contains quite a quantity of green pigment. It is in the form of small spherical granules, and is scattered only sparsely over the surface. There seems to be great variation in the amount of pigment which may be found in the larvae. In many individuals it seems to be entirely absent, and its place is supplied by small spherical particles of highly-refracting substance (Fig. 21, *p.*), which seem to be the pigment-granules which have lost their pigment. The abundance of pigment seems to be dependent to a certain degree upon nutrition, since it is always more abundant in well-fed larvae than in those whose food is scanty.

Besides these structures, there is another system of organs belonging to the ectoderm. Very early in the development of the larva the body is seen to undergo considerable contractions, which must be due to muscles. They are seen long before the true mesoderm muscles are developed, and even after their development it is plainly seen that the ectoderm is chiefly concerned. They are due, undoubtedly, to certain muscles which can be seen by examining the ectoderm closely with a high power (Fig. 21, *e. m.*). They lie wholly in the ectoderm, and undoubtedly originate in this layer, although I have not been able to demonstrate very satisfactorily the method of their development.[1] By using Hertwig's teasing solution, two or three

[1] Hertwig's Actinien, Jen. Zeit. XIII.

different kinds of ectodermal cells can be distinguished (Figs. 37 and 39). Some of them (Fig. 39) bear much resemblance to the well-known epithelio-muscular cells of coelenterates, but the difficulty of teasing such small animals makes it almost impossible to get satisfactory evidence. That the muscles are ectodermal I have no doubt, but whether they arise as epithelio-muscular cells, as in the coelenterata, is not so certain. Stossich has described ectodermal muscles in Serpula,[1] but his evidence is hardly sufficient, and his observations have not been confirmed.

Alimentary Tract.

The alimentary tract consists of three divisions, oesophagus, stomach and intestine. The mouth lies upon the ventral surface of the larva between the two ciliated bands (Figs. 27 and 28). It is an oval opening situated in the midst of the broad tract of cilia found between the ab-oral and pre-oral ciliated bands. The cilia serve for collecting food, which consists mostly of microscopic algae.

The mouth leads into the oesophagus, which is a long, narrow tube projecting towards the anterior end of the body, and nearly reaching the supra-oesophageal ganglion (Fig. 27, *oe.*). Its opening into the stomach is upon the dorsal side (Fig. 27, *o.*). The oesophagus consists of high columnar, ciliated cells. To aid the cilia in carrying food to the stomach are a number of muscles connecting the oesophagus with the body-wall, which, by their contractions, can very much enlarge the cavity of the oesophagus (*mc.*). These muscles are of great importance to the larva, for its powerful cilia frequently force into the mouth particles of food so large that the cilia cannot propel them into the stomach. The muscles, then, by contracting, enlarge the diameter of the oesophagus, and thus facilitate the passage of the food.

The central portion of the digestive tract, the stomach, is much the largest of the three, in its fully-expanded condition nearly filling the body-cavity (Fig. 27, *st.*). From the first it is quite different in appearance from the oesophagus, having much thinner walls and being more contractile, at times when not distended with food shrinking to small compass. It is composed

[1] Stossich. *Loc. cit.*

of cells which are quite small and form a thin, flat layer. When separated by teasing while fresh, they assume a spherical form and appear as ciliated cells, each nucleated, and containing a number of highly-refracting bodies, evidently of an oily nature, and being the result of food-digestion (Fig. 38). One region of the stomach is particularly set apart to secrete the digestive fluids. If a section be cut longitudinally through the larva, it will be seen that the stomach-wall directly beneath the oesophagus is composed of cells not as thin as the ordinary cells, but very large, and forming a thick endodermal ridge (Fig. 40). The cells are highly granular, possess a very distinct nucleus, and have every appearance of glandular cells. This endodermal ridge cannot be seen in the living animal, and it is only in section that it becomes visible. Fig. 40 shows the ridge with its great cells graduating at either end into the ordinary endodermal cells.

The whole of the stomach is lined with cilia, which keep the food in constant rotation. The cilia are mostly short, except at a ridge around the opening of the oesophagus. Here (Fig. 27, *co.*) we find a crown of long, powerful cilia, whose object is to effect the introduction of food.

The intestine, composed of ciliated columnar cells, does not open into the stomach at its posterior end, but upon its ventral side, as shown in Fig. 27. Its length is subject to great variation during the growth of the larva.

Connected with the digestive tract is a peculiar structure the significance of which is questionable. This is a thickened ciliated ridge of a more or less complex form, which appears early in the larval history. It begins upon the right side of the stomach close to the mouth, and after one or two turns (Fig. 27) runs upon the intestine and follows this upon the ventral median line towards the anus and disappears. As the development goes on, the ciliated ridge becomes more developed with the development of the alimentary canal, and finally becomes very contorted. Upon close examination it appears to be a ciliated tube. This organ, first described by Salensky[1] in his Echiurus larvae, was considered by him to be respiratory in function. Hatschek[2] also figures and describes it, but offers no

[1] Über die Metamorphose d. Echiurus, Morph. Jahr. II. [2] *Loc. cit.*

suggestions as to its function. In the oldest larvae that I have seen it was still a prominent structure, although rather difficult to observe on account of the opacity of the animal. I would suggest that it may be the beginning either of a peculiar ciliated groove which is seen in the adult, occupying the ventral surface of the stomach and intestine, or the beginning of a secondary gut which, in the adult, accompanies the alimentary canal. Nothing of the kind is found in the quite similar larva of Serpula.

Mesoderm.

The mesodermal formations in Thalassema are twofold: 1. A mesodermal system very similar to that in Echinoderms and Mollusks, which is called by the Hertwigs, in their "Coelentheorie," the mesenchyme; 2. A true mesodermal system like that of other annelids.

In echinoderms the mesenchyme arises as follows:[1] The segmented egg gives rise to a single layer of cells surrounding a large segmentation-cavity. Into this segmentation-cavity are budded off from the external layer a number of cells. They first appear at the position of the future blastopore, and are products of that portion of the embryo which is immediately to be invaginated to form the endoderm. The cells thus appearing wander about in the segmentation-cavity, multiply rapidly, and eventually the entire cavity is filled with these wandering cells, which form the mesoderm or mesenchyme of the adult.

Inasmuch as some of the mesodermal muscle-cells in the Thalassema trochosphere bear so complete a resemblance to a mesenchyme, one would naturally expect to find a similar origin. Indeed, the Hertwigs have no hesitation in deciding, upon histological grounds, that there is a true mesenchyme. A study of the early history of the larva shows that there is some difference in detail, although the essential features are the same. Neither before the invagination nor during the gastrula period are these cells differentiated. Some time later they appear as cells budded off from the endodermal mass, most of them arising quite near the blastopore. They do not, however, become true wandering cells, but are immediately transformed into muscles connecting

[1] Selenka. Zur Entwicklung der Holothurien, Zeit. f. Wiss. Zool. XXVII.

the oesophagus with the body-wall, whose duty, as already mentioned, it is to enlarge the cavity of the oesophagus, and to control its movements. They form two sets—the first connecting the oesophagus with the body-wall (Fig. 27), and the second connecting the oesophagus with the stomach *mc.'*. They have no very great regularity of distribution in different individuals, but we always find a number attached to the ectodermal thickening of the nervous system; and there is one muscle which is universally found connecting the oesophagus with the stomach upon its dorsal surface (Fig. 27 *mc.'*). Each muscle is unicellular, consisting of a cell-body with a distinct nucleus, and with muscle-fibres extending from it in two directions (Fig. 25). The muscle-fibres may occasionally be branched, but the branching is very simple, and is always in that part of the fibre connecting the cell with the ectoderm, and not in that part joining the oesophagus (Figs. 24–26).

Besides these distinctly muscular cells, there are a large number of mesodermal cells whose origin is partially, at least, the same. They are branching cells, like connective-tissue corpuscles, and are to be seen in all larvae, scattered somewhat at random in the body-cavity, but quite close to the body-wall. In the older larvae they are more abundant, and unite to form quite a continuous layer.

In most trochosphere larvae there is a large, prominent muscle uniting the supra-oesophageal ganglion with the oesophagus. In Thalassema no such muscle is found, but a muscular band, which is probably its homologue, is seen extending from the nervous thickening to the region of the pre-oral cilia (Fig. 27, *vlm.*), and may sometimes be traced beyond this in the post-oral lobe, *vlm.'*. We do not find, as is usual, a distinct muscle or a muscular bundle, but a broad band of separate muscular fibres loosely arranged and closely adhering to the body-wall. Upon the origin of the muscle I have made no observations, except that it appears with the completion of the trochosphere.

The first division of the mesodermal system is thus in its origin very like the mesenchyme of Echinoderms and Mollusks, being budded off from the endodermal cells as wandering cells. Like the mesenchyme of these groups also, it gives rise to (1) a quite complex muscular system made up of independent muscular

fibres, and (2) a large number of cells which do not appear to become converted into muscles.

The second division of the mesoderm, the true mesoderm, is in the early stages only very slightly developed. It is seen in the shape of two small bands of cells near the anus. Owing to the opacity of the larva, I could not make out definitely their origin, but, judging from what occurs in Serpula, I conclude that they arise from the endodermal cells of the intestine. They bear a close resemblance to the same structures in other annelids.

VI.—*Later Stages.*

The stage of the complete trochosphere larva, as above described, is reached in about three days. From this time onward the growth is much slower, since for nutriment the animal is obliged to depend upon the prey it may capture, rather than upon the store laid up for it in the egg. I have, however, had no difficulty in keeping the larvae alive until they had assumed nearly all of the adult characteristics. I have therefore been able to witness the entire development from the egg to a stage almost adult, and make careful study of each stage. All of the later larval history of Echiurus has been already carefully studied and described by Hatschek, and, inasmuch as the development of Thalassema is almost exactly the same as that of Echiurus, I shall not attempt to give a detailed account of the later history, but simply an outline, referring for further details to the paper of Hatschek.[1]

The history of the larva from this time on is that of a continuous growth with nothing to divide it into separate stages, until quite a remarkable change takes place just before the adult condition is assumed. The most noticeable feature is a slight change in the shape of the larva. In the trochosphere which I have described, the pre-oral lobe equals or often surpasses the post-oral lobe in size; but this relation is soon reversed, so that after the fourth or fifth day the post-oral lobe is the longer. But right here do we come to the chief difference between Thalassema and the ordinary annelid. The post-oral lobe does become longer than the pre-oral lobe, but not very much longer. In

[1] Arb. a. d. Inst. Wien, III.

other annelids which pass through a trochosphere stage the post-oral lobe grows out to form a very long body, while the pre-oral lobe remains very small (the cephalic segment). Here, however, the pre-oral lobe very nearly keeps pace with the post-oral lobe in its growth, so that in the adult the pre-oral lobe or proboscis is as long as the rest of the animal. At this point, therefore, do the Echiuridae branch off from the annelids, they on the one hand retaining a large pre-oral lobe, while the annelids develop the post-oral lobe at its expense; the former group also developing a long alimentary canal, while the latter develop segments.

The general external changes in the later history are very simple. A second band of cilia makes its appearance, consisting of a single row just in front of the anus—a peri-anal band. As the animal grows, it increases in length more than in breadth, and gradually thus becomes more and more worm-like. The post-oral lobe acquires a great amount of mobility, and is found in very different states of contraction, sometimes extended as in Fig. 42, or at other times so much contracted that the animal is nearly spherical. When swimming through the water freely, it is in a state of extreme expansion. As the intrinsic motions due to muscles become of more importance, the ciliary motions become less noticeable, and finally, when the stage of Fig. 42 is reached, the cilia have become of minor importance. The animal stays most of the time on the bottom, though still able to swim slowly. Finally, the cilia disappear altogether (Fig. 43), and the only means of locomotion which the larva possesses is by slowly crawling by its muscular contractions. All of this time, however, the larva still remains a trochosphere, differing from the younger stages only in the slightly greater length of the post-oral lobe.

Leaving for the present the external changes, I will briefly describe the internal changes which have in the meantime taken place. The most important of them all, and indeed one of the most important features in the development of the animal, is the history of the mesodermal bands. We have already seen these in the trochosphere as small masses of cells (Fig. 27) lying near the anus, one on either side of the median line. As the development proceeds, they gradually grow forwards, arching outwards

slightly, and soon become thus two narrow bands of cells lying on either side of the ventral median line, somewhat broader in front than behind—precisely, in fact, as in ordinary annelids. Not only do they thus resemble annelids in their early history, but in about three weeks after the fertilization (time varying widely with the food) they become segmented in a perfectly normal manner (Fig. 52), so that the young larva of this period is a typical annelid larva (Fig. 42). The segments are quite numerous and very distinct. The segmentation is also shared by the nervous system, which, when formed, has one ganglion for each segment connected by a narrower band (Fig. 43). This segmentation, first described by Hatschek, is quite a remarkable fact, and indicates conclusively that the Echiuridae are nearly allied to annelids.

I next notice the completion of the nervous system. It consists at first of the ectodermal thickening above described, which gives rise to the supra-oesophageal ganglion. Of the ventral ganglion the only indication is the band of cilia upon the ventral line, present from the earliest stages to the very latest stages of the larval history. About the same time with the segmentation of the mesoderm the true ventral nerve-chord begins to be formed. Sections of the larva at this period indicate that the chord arises in a manner almost precisely like that of Lumbricus.[1] It is developed from the ectodermal cells at the base of the cells which bear the above-mentioned cilia, arising probably from these cells. It is formed as two chains of separate ganglia. Fig. 41 is a section through the ventral surface, showing the ventral chord at the time of its appearance. In the middle are seen the ciliated cells, while at their bases on either side are small cells which differ markedly from all of the other cells of the ectoderm. These boundaries are distinct, showing them to be spherical, and each possesses a well-defined nucleus. That they are segmented is easily seen a little later, when the whole chain becomes visible externally. Fig. 43 shows the ventral chord, remarkably large and distinct, and with a separate pair of ganglia for each segment.

Quite late in the larval life this chain becomes connected with the cerebral ganglion by two long commissures, whose origin is

[1] Kleinenberg. Quar. Jour. Mic. Soc. XIX.

also ectodermal, but the precise method of their formation I have not made out.

Some time after the mesoderm has become segmented we find the first traces of the ventral setae. These two setae are situated one on either side of the median line, just posterior to the mouth, and form the only armament which Thalassema possesses. A study of their adult structure would lead us to conclude that they are of ectodermal origin, formed by ectodermal ingrowths. But such is not, however, the case. When first appearing they are seen to be formed in small mesodermal sacs, entirely beneath the ectoderm and unconnected with it. They rapidly elongate, and soon force their way through the ectoderm and come thus to project upon the outside (Fig. 42). Meantime muscle-fibres become connected with their internal ends, which thus connect them with the body-wall. The muscles radiate in all directions, so that the setae can be moved at will.

The digestive tract has in the meantime been constantly growing in length, at first somewhat slowly, but afterwards more rapidly. For a long time this growth affects the intestine only, which becomes considerably contorted, as can be seen by the increasing complexity of the ciliated furrow. Later the stomach also begins to elongate and become folded, until finally a long, convoluted digestive tract, such as is represented in Fig. 43, is attained, much longer than the body. All this time, and even in the adult, the separation into oesophagus, stomach and intestine remains distinct. The region where the oesophagus and the stomach join is always somewhat swollen, and it remains so in the adult, forming a short section of the digestive tract known as the crop.

Quite late in the development, after all the changes above mentioned have taken place, appear two structures known in the adult as anal pouches. In the adult they appear to be diverticula from the alimentary canal, but a study of their development shows this not to be the case. They arise as ectodermal invaginations quite close to the anus, but separated from it (Fig. 42). Later they approach more closely to the anus, and finally the openings are carried within it, giving the relations as seen in the adult. But the fact that they are ectodermal in origin is of considerable importance in understanding their morphological meaning.

Thus far my own observations are little more than a corroboration of those of Hatschek. Indeed, in every important point except one I find that Thalassema agrees with Echiurus. I have, however, been unable to discover any traces of the peculiar excretory organ so minutely described by Hatschek in Echiurus. This has been a great surprise to me, and I have consequently searched long and carefully for it; but at no stage of the development have I found it possible to discover any trace of it, either by section or in the living specimen. Whether this indicate that the primitive kidney is not present in Thalassema, or that the various ectodermal structures entirely mask it and prevent its being seen, I will not attempt to say. I do not think it probable that I could have overlooked so prominent a structure, if it were present.

The larva as now described, though still a trochosphere, has present in rudiment all of the adult structures with the exception of the vascular system and the sexual organs. But now quite a remarkable change takes place, which may almost be called a metamorphosis. The anal pouches are simply blind invaginations at first, but they have now increased somewhat in length and acquired an internal opening into the body-cavity. This funnel-opening is shown in Fig. 43. As soon as this happens the animal rapidly absorbs water, taking it in probably through these openings. The result is that the larva changes its appearance remarkably. Hitherto (Fig. 42) it has possessed a power of locomotion by means of its cilia, though usually remaining on the bottom. It has been quite opaque, owing to the numerous pigment-bodies, and has been visibly divided into segments. When it begins to absorb water, however, in a few hours its appearance is entirely changed (Fig. 43). It increases nearly four times in bulk, owing chiefly to the body-cavity being expanded by the absorbed water. The pre-oral band of cilia entirely disappears, and the animal can only crawl over the bottom by means of its pre-oral lobe, which has increased its flexibility and power. The pigment begins rapidly to disappear, and all visible evidences of segmentation are completely gone. The animal has consequently become perfectly transparent, with the exception of the pre-oral lobe, which does not seem to share in the absorption of water, and now becomes even more opaque

than ever by the multiplication of its muscle-cells. Quite a similar absorption of water has been described in Bonellia by Spengel.[1]

After this metamorphosis the larva is a remarkably different animal (Fig. 43). An examination of the transparent animal indicates that it is in almost every respect adult. The post-oral lobe has now become clearly differentiated as the body, while the pre-oral lobe is the proboscis. As yet, however, the digestive tract extends for some distance into this lobe, but gradually its muscle-cells multiply and begin to fill its cavity. The digestive tract is thus expelled from the pre-oral lobe, which becomes a solid, prehensile organ of the adult. The nervous system is now complete, the supra-oesophageal ganglion being now united with the ventral chord by two long commissures. The ventral chord, however, still retains its segmentation, and, indeed, does so for a long time. The two ventral setae have their adult structure and position. The anal pouches are now closely connected with the alimentary canal at the anus. As yet they have only a single internal opening, which is undoubtedly the terminal opening of the adult. The various scattered mesenchyme-cells of the post-oral lobe (Fig. 27) have given rise to a system of muscles connecting the digestive tract with the body-wall-muscles which are very numerous and very important in the adult. Finally, many of these mesenchyme-cells have become free and float around in the body-cavity as blood-corpuscles. They are at first few in number, but gradually increase, seemingly by division, although of this point I am not positive.

The larval history is now essentially completed, since a form is reached which is nearly adult. The animal can no longer swim, and is only able to crawl very slowly. Probably this is the stage at which the young Thalassema enters its final home in the sand-dollar shell. Here it goes through the few final changes which are needed to make it a sexually mature animal. The alimentary canal continues to increase in length, finally becoming very long—20 times the length of the body. The muscles of the pre-oral lobe continue to multiply until this member becomes solid. The anal pouches acquire other openings

[1] Spengel. Ent. der Bonellia. Naples Mittheil. Vol. I.

similar to the terminal one shown in Fig. 43. The vascular system appears probably in the mesoderm. Finally, the sexual pouches arise as ingrowths from the ectoderm, and the mature condition is reached.

VII.—*General Conclusions.*

Summary.

One of the cells of the peritoneal lining of the body-cavity takes upon itself certain powers the significance of which, in the present state of biological science, we little understand, and which we are only able to define by the effects produced. This cell, which previously differed, as far as can be seen, not at all from the other cells of the peritoneal membrane, becomes an ovum, a separate, independent individual, and soon loses its connection with the body of the mother, and floats around freely in the peri-visceral fluid. It rapidly picks up nutriment from this fluid, using part of it to increase its bulk and stowing part away in the form of yolk-granules for future use. In this way it increases many times its original size, and finally finds its way into the sexual pouches. Here it remains in a state of inactivity, but ever ready for further development the instant it is placed in the right circumstances. The period during which it remains thus at rest varies somewhat, being longer for eggs produced early in the summer than for those appearing later; but finally the entire contents are discharged from the body, and from this time each egg floats independently in the water.

It is now fertilized by a spermatozoon which has had a history quite similar to that of the ovum, but in another individual. Fertilization is followed by the protrusion of two polar globules, when follow a regular segmentation and an invaginate gastrula. Very early the ectodermal cells opposite the gastrula mouth become thickened to form the beginning of the nervous system. The gastrula stomach elongates, becomes divided into three divisions by constrictions, bends upon itself, and finally unites with the body-wall, and the anus breaks through at a place which corresponds to one end of the blastopore, which has in the meantime become elongated. Cilia make their appearance, at first as a complete covering, then becoming constricted to a broad

band in front of the blastopore, and finally taking the form of two definite rows of large locomotor cilia, one pre-oral and one post-oral. Later, a third, a peri-anal ring, is developed. The larval form thus reached is a typical trochosphere. This larva continues for a long time free, leading an active life and growing in size and complexity. The second part of the nervous system makes its appearance as a ventral ectodermal thickening, occupying thus the position of the closed lips of the lengthened blastopore. The two parts of the nervous system are thus at first completely separate. The muscular system arises from two mesodermal bands appearing near the anus, but growing forward and becoming distinctly segmented. Now, by a continual growth, the adult form is reached. The segmentation disappears, the pre-oral lobe becomes filled with muscular tissue, the setae appear as mesodermal organs, the anal pouches arise as ectodermal invaginations, and finally admit through their internal opening a large quantity of water into the body-cavity, which causes the animal to increase much in size.

How long a time elapses between the first stages of development and the close of free existence is not known. It varies with conditions, food, temperature, etc. Finally the larva finds its permanent home in some cavity in a sand-dollar shell. Here, by means of its pre-oral lobe, which has now become long, flexible and muscular, and by the aid of secretions from dermal glands, it arranges for itself rough chambers in the sand with which the shell is filled. In this chamber it remains a prisoner. Here it grows to maturity, completely protected from external attack, and finding sufficient food in the small amount of organic matter adhering to the sand, which it passes through its alimentary canal in great quantities. Its only means of communication with the exterior is through the small oral opening of the sand-dollar shell, and through this it must obtain all its food and cast its sexual products when mature.

The development of Thalassema and Echiurus, taken together with their adult anatomy, enables us to determine decisively the relations which they bear to other worms. The Echiuridae are undoubtedly highly modified annelids. Comparing their development with that of other annelids, we can come to no other conclusion. The early stages of very few annelids have been studied,

and in most cases the line of development has been modified by secondary causes (food-yolk, etc.), to enable us to make out the true history. But, with the appearance of the trochosphere, the two groups can be closely compared. Compare Thalassema trochosphere with Polygordius,[1] or with Serpula, and the resemblance, even to points of histological detail, will be found to be very striking. The further development shows, likewise, the same mesodermal bands, which give rise in each case to a *segmented* mesodermal system. In both groups we find the same double origin of the nervous system, the cerebral ganglion appearing very early at the anterior end of the larva, and the ventral chord arising later as a series of paired ectodermal thickenings.

From the trochosphere the two forms soon, however, branch off from each other and take slightly different lines of development. In ordinary annelids the post-oral lobe elongates greatly, while the pre-oral lobe is relatively much reduced. In the Echiuridae the elongation of the pre-oral lobe nearly equals that of the post-oral lobe. In the ordinary annelid the pre-oral lobe becomes the head-segment, containing the cerebral part of the nervous system, and having the mouth at its ventral posterior limit. In Echiuridae it becomes the pre-oral lobe, with like relations to nervous system and mouth. In ordinary annelids the segmentation remains as an adult characteristic. In Echiuridae it is only an embryonic feature, and soon disappears.

There can be no doubt, then, that the Echiuridae are annelids. It is evident, also, that their relation to other annelids is not that of a primitive type, but of a highly-modified form in which, for some reason, the segmentation has been lost. Polygordius stands at one extreme, the Echiuridae at the other. It is hardly safe to speculate as to the reason for the loss of segmentation, but we can at least go so far as to say that it probably arose in connection with the elongation of the alimentary canal, and was, indeed, perhaps caused by this, since the lengthening of the canal would of necessity cause the disappearance of internal segmentation. With this modification, other changes in adult

[1] Agassiz. On Young Stages of a Few Annelids. Annals of Lyceum of Nat. History, New York, Vol. VIII, 1866. Also Hatschek. Arb. a. d. Zool. Mit. Wien, Vol. I.

anatomy have arisen. The setae have disappeared, with the exception of a single pair. The segmental organs have become much changed. Two pairs in the anterior part of the body become sexual pouches, and a single pair become very highly developed, and fill the function which the whole system originally filled. These are the so-called anal pouches. All the rest have disappeared. The body-cavity, being no longer divided into chambers, and being filled with a peri-visceral fluid, makes the demand for a circulatory system of less importance, and we consequently find this system has become much reduced in extent, and is confined chiefly to the pre-oral lobe, into which the body-cavity and peri-visceral fluid do not extend. And all of these changes we can easily understand as the result of the loss of segmentation.

Thalassema and Echiurus must therefore be regarded as annelids which have lengthened their alimentary canal and simultaneously lost their segmentation. Bonellia, though classed with the Gephyrea armata, differs so much in its development from these two forms as to make it at least doubtful whether there is any close relation between them.[1] These differences may, perhaps, be partially explained by the fact that, while Thalassema has a perfectly free embryo from the beginning, and consequently an ovum with its food-yolk uniformly distributed, Bonellia has a stationary ovum, and one in which the food-yolk is massed so as to give rise to an epibolic gastrula. The other so-called Gephyrea differ so much from the Echiuridae, both in adult anatomy and in embryology, as to make it certain that their relations are very distant, and that there is no propriety in classing them together in any one group.

EXPLANATION OF PLATES.

PLATE XX.

FIGURE 1.—Unfertilized ovum.

FIGS. 2–4.—Extrusion of first polar globule.

FIG. 5.—First resting period.

FIGS. 6–7.—Extrusion of second polar globule.

[1] Spengel. Beiträge z. Kenntniss d. Geph. Mit. a. d. Zool. Stat. zu Naples, Vol. I.

FIG. 8.—Division of first polar globule.

FIG. 9.—Second resting stage.

FIGS. 10–12.—Segmentation.

FIG. 13.—Showing striated structure of gelatinous mass between ovum and vitelline membrane.

PLATE XXI.

FIG. 14.—Third resting stage of ovum.

FIG. 15.—Optical section of ovum, with thirty-two segments, showing segmentation-cavity.

FIG. 16.—Section through gastrula.

FIG. 17.—Embryo showing stomach and oesophagus distinctly separated. Partially an optical section.

FIG. 18.—Older embryo from side. *bl*, blastopore.

FIG. 19.—Embryo of twenty hours showing first appearance of mesenchyme-muscles.

FIG. 20.—Slightly older embryo from the dorsal side, showing appearance of constrictions to separate stomach from intestine.

FIG. 21.—Ectoderm of trochosphere, showing *gl*, ectodermal glands; *em*, ectodermal muscles; *p*, pigment-granules.

FIG. 22.—Showing cerebral nervous thickening from the side.

FIG. 23.—The same in the dorsal view.

FIGS. 24–26.—Fully-developed mesenchyme muscle-cells.

PLATE XXII.

FIG. 27.—Full-grown trochosphere in optical section. *m*, mouth; *o*, opening of oesophagus into stomach; *an*, anus; *cc*, long cilia guarding oesophageal opening into stomach; *cr*, ciliated ridge; *mb*, mesodermal bands; *mc*, mesenchyme muscles; *mc*, muscle uniting stomach and oesophagus; *oe*, oesophagus; *po*, pre-oral ciliated ring; *pro*, post-oral ciliated ring; *st*, stomach; *vc*, ventral cilia; *vlm*, longitudinal muscular band.

FIG. 28.—Same larva in surface view.

FIG. 29.—Optical section through ciliated region near mouth.

FIG. 30.—Ectoderm after treatment with osmic acid.

FIG. 31.—Same before treatment.

FIG. 32.—Optical section of ciliated region near dorsal surface.

FIG. 33.—Optical section through posterior end of the body.

FIG. 34.—Section through oesophagus.

FIG. 35.—Ciliated band teased in acetic acid.

FIG. 36.—Ciliated region showing large cells from which the cilia arise.

FIG. 37.—Ectodermal cells.

FIG. 38.—Endodermal cells.

FIG. 39.—Epithelial muscle-cell (?).

FIG. 40.—Section through stomach, just below the oesophagus, showing gland (?) cells.

PLATE XXIII.

FIG. 41.—Section through ventral part of body showing origin of a paired nervous chord.

FIG. 42.—Larvae several weeks old.

FIG. 43.—Same a few hours later, after the absorption of water.

FIG. 44.—Section of testis.

FIG. 45.—Groups of spermatozoa mother-cells floating around the body-cavity.

FIG. 46.—Groups of developed spermatozoa clinging together and floating in the body-cavity.

FIG. 47.—Section of ovary. *ov*, ova; *n*, nuclear bodies.

FIG. 48.—Group of ova floating in body-cavity.

FIG. 49.—Highly-magnified section of the edge of an ovum, just before it enters the sexual pouches, showing peculiar striated surface.

FIG. 50.—Section of mature ovum from sexual pouch.

FIG. 51.—Section through a sexual pouch, showing its opening into the body-cavity, guarded by the upper lip, *ul*, and lower lip, *ll*, and the external opening, *eo*, to the exterior.

A CONTRIBUTION TO THE EMBRYOLOGY OF THE PROSOBRANCH GASTEROPODS. By J. PLAYFAIR McMURRICH, M. A., Ph. D., Instructor in the Johns Hopkins University, Baltimore, Md. With Plates XXIV, XXV, XXVI, XXVII.

In the following pages I propose giving an account of certain features in the development of some of the marine Prosobranchs, and of the theoretical considerations suggested thereby. The material upon which my observations were made was collected during last summer at the Marine Laboratory of the University at Beaufort, N. C. Certain observations were made upon the fresh material while I was at the seaside, but all section-cutting, etc., was performed during the past winter on material preserved, for the most part, by treatment with corrosive sublimate and alcohol. Perenyi's fluid was also employed, but the results were not satisfactory, the reagent causing excessive swelling and distortion of the eggs containing much yolk-material.

Fulgur carica formed the principal subject of investigation. The egg-capsules of this species are well known and will not require description here. *Fasciolaria tulipa*, var. *distans*, was also the subject of study on some points, its triangular capsules being very common on the mud-flats in front of the Laboratory. In the same locality were to be found, fastened to stones, etc., in large clusters, capsules (Pl. XXIV, Fig. 2) of a columnar shape, flattened upon one side, truncated above, and tapering slightly below, where they unite with a parchment-like expansion covering the surface of the object upon which they are deposited. They are about 15 mm. in height, and in all clusters of large size certain of them were of a more or less decided pink color. On two or three occasions I found in connection with them specimens of *Purpura floridana*, but could not satisfy myself whether they were there for ovoposition or for predatory purposes. Mr. Geo. W. Tryon, Jr., the well-known conchologist, in reply to my inquiries, kindly sent me sketches of the capsules of a species of *Purpura* (not *floridana*) which bear some resemblance to those concerning

which I desired information, and like them many presented a pink coloration. Mr. Tryon believes there can be little doubt but that the capsules under discussion belong to *Purpura floridana*, and I shall in the following pages speak of them as belonging to that form.

Crepidula fornicata, plana and *convexa* were observed to a slight extent. They deposit their ova in capsules, which are retained beneath the shell of the parent, and are in consequence delicate and not parchment-like, as in most of the other marine Prosobranchs. They form a number of irregularly shaped pouches attached at nearly the same spot to the shell of *Limulus*, or whatever it may be on which the parent lives.

I also obtained a few capsules in the trawl, which Professor W. H. Dall has allowed me to compare with some unpublished sketches of his own, and which I have thereby been able to identify as belonging to *Eupleura caudata*. The number of these capsules obtained was very small, and I was only able to ascertain from them the general plan of segmentation which the ova follow.

I.—THE OVUM AND THE NUTRITION OF THE EMBRYO.

Each capsule of *Fulgur* contains only a few eggs, the number averaging about 12 or 14. The eggs are exceedingly large, and somewhat oval, measuring 1.5 mm. in their long and 1 mm. in their short diameter. They are imbedded in a large quantity of albuminous material, and contain a very large amount of yolk, whence results their extraordinary size. The yolk consists of numerous round and oval granules, varying in size from .004 mm. to .07 mm. in diameter, closely packed together. The chemical nature of these granules seems to be entirely unknown, very little work having been done, in fact, on the eggs of any forms except the Vertebrata. The brief account given in Watt's Dictionary of Chemistry of the observations of Valenciennes and Frémy on the molluscan egg would lead one to conclude that these investigators had studied the albuminous substance in which the eggs are imbedded instead of the egg-yolk itself, and had, in fact, mistaken the egg-capsule for the egg. My own

observations are not at all extensive, but have sufficed to show the proteid nature of the granules. With Millon's reagent they give the characteristic pink color, and with nitric acid the bright yellow coloration even without the addition of ammonia or caustic potash. Granules which have been treated with corrosive sublimate and alcohol are quite insoluble, but unfortunately I cannot state whether or not they are soluble before this treatment. Warneck, however, according to Fol (11), states that in unfertilized eggs they are insoluble when the egg is crushed in water, but after fertilization are soluble under the same conditions, and argues therefrom the occurrence of a profound chemical change of the food-yolk as a result of fertilization. All these facts tend to establish the proteid nature of these granules. Blochmann (2) describes in the ova of *Neritina fluviatilis*, Müll., large yellow globules of food-yolk. The smaller of these he states are soluble in ether (also in alcohol), while the larger ones are unchanged. He concludes, therefore, that the smaller food-globules are of a fatty nature. With the granules of *Fulgur* I obtained no such reaction, neither the large nor the small granules being altered in any way by alcohol, ether or chloroform, and I have no doubt but that all, both large and small, are of the same nature. One interesting reaction I may mention, although not of chemical importance. From their general appearance I at first considered that they might possibly be amylaceous, and tried the effect of Schultz's chlor-iodide of zinc. By it they were stained yellow, and at the same time were differentiated into two portions, a central portion less deeply colored, and an outer or peripheral portion of a brown color. In small granules (Fig. 1 *a*) the central portion is quite small, the peripheral portion constituting the greater part of the granules, but in larger ones (Fig. 1 *b* and *c*) the reverse is the case, the peripheral being in the largest granules almost reduced to a membrane surrounding the large, light-colored central portion. This may be due to the action of corrosive sublimate which the granules had previously undergone. In some respects these molluscan yolk-granules remind one of the so-called amylaceous bodies found in certain flagellate Infusoria, such as *Euglena* and *Astasia*. My own observations (20) of these structures seemed to show that they were not amylaceous, but

were not carried far enough to yield any positive results. It is quite possible that further investigations may prove them to be proteid, and very similar, therefore, to the granules in the molluscan egg. A study of the food-yolk of the ova of various invertebrate groups would, it seems to me, yield results of no little interest and perhaps importance.

In *Fulgur* all the eggs contained in each capsule develop, but this is not the case in some other forms I have examined. The egg-capsules of *Purpura floridana* are, when newly deposited, white or slightly yellowish, but after a few days become of a pinkish hue, which increases in intensity as development proceeds, until the capsules become of a deep magenta color. This was found to be due to a change in color not of the capsule itself, but of its contents. Numerous eggs measuring 0.096 mm. are contained in each capsule, and all undergo segmentation; but as segmentation proceeds many break down, their yolk-granules assuming the magenta hue spoken of above, and are devoured by the embryos which have not undergone this disintegration, so that they become filled with magenta-colored granules, their provisional excretory cells also assuming the same hue. The breaking down and disintegration of the ova commence at a comparatively early stage, in some cases as early as the fifth or sixth stage of segmentation—*i. e.*, when one embryo consists of four macromeres and twelve or sixteen micromeres.

In *Crepidula fornicata*, *plana* and *convexa* each pouch contains a comparatively large number of eggs, all of which seem to be at much the same stage of development. A few of the ova apparently break down, as in *Purpura floridana*, and are used as food by the surviving embryos; but the process is not so evident as in *Purpura*, nor do the food-granules assume a magenta color.

An extreme case of the same phenomenon is exhibited in *Fasciolaria tulipa*, var. *distans*. Each egg-capsule of this form contains a large number, perhaps two hundred, of ova, measuring about 0.25 mm., imbedded in the usual albuminous substance, and containing much yolk. Of these, however, only four or six ever develop, the rest being swallowed by the developing embryos and used as food. The non-developing eggs do not even segment nor push out polar globules, nor do they break down or dis-

integrate, but are ingested entire by the embryos, so that at an early stage one of these appears to consist of a number of ova bound together into a ball furnished with a velum provided with two large provisional urinary bodies on its lower surface.

Similar phenomena have been observed in other molluscan ova. Selenka has described for the Opisthobranch *Tergipes* (26) a segmentation which proceeds without any regularity whatever, some of the spherules, in fact, being thrown off and becoming quite independent of the ovum. These develop cilia and move about in the interior of the egg-membrane, forming Von Nordmann's parasitic "Cosmellae." This phenomenon, however, can hardly be considered normal, and it is not stated that these separated spherules are ingested by the embryo so as to be of use in its development. Koren and Danielssen (16) describe a process much more to the point in *Buccinum undatum*, in which they describe a conglomeration of numerous ova to form a single embryo, some of the ova, however, developing independently for a time, but eventually degenerating and dying. A similar process also occurs in *Purpura lapillus*. Carpenter (10) investigated the development of this latter form, disputing the views of Koren and Danielssen, and concluding that of the 500–600 eggs in each capsule only 12–30 which develop are true ova, the rest being yolk-spherules, which divide without any regularity into 14 or 20 segments, and are then swallowed and used as nutriment by the developing embryos. Selenka, much later (27), also studied *Purpura* and recognized the correctness of Carpenter's observations. He does not regard the segmentation of the sterile eggs as equivalent to that of the fertile ones, on account of its irregularity, its absence in some cases, the absence of a nucleus in these eggs, and its presence in the fertile ones (and not the reverse, as Fol (11) states in the brief abstract he gives of Selenka's paper), and the similarity of the spherules to the food-yolk of the fertile ova. In *Neritina fluviatilis*, of the 70–90 ova contained in each capsule only one develops to maturity; the remainder being used as nutriment. Blochmann (2) shows that in all the ova in each capsule the polar globules are excreted with the usual karyokinetic changes in the nucleus, and the female pronucleus is formed in the normal manner; but subsequently to these processes a difference can be noticed, the single

fertile egg segmenting regularly according to the usual Gasteropod type, while in the sterile ova no such regularity can be perceived, although they too segment. Blochmann argues that the regular segmentation of the fertile egg is due to the penetration into it of the male pronucleus, and that the sterile eggs are unfertilized. In this he agrees with Bütschli (9). Lastly, Brooks (5) states that all the 6–20 eggs contained in a capsule of *Urosalpinx* normally undergo development, but occasionally a partially segmented egg or more advanced embryo breaks down and is used as nutriment by the survivors; "but, while this method of furnishing the young with food appears to be normal with *Purpura lapillus*, it is accidental and exceptional with *Urosalpinx*."

This last case seems to afford a clue to the manner in which the phenomena seen in *Fasciolaria*, *Purpura lapillus*, etc., have been brought about. An occasional egg in a capsule has from some cause or other broken down, and has been drawn into the digestive cavity of the developing embryos. This process, having proved useful, is continued, and an arrangement such as I have described above for *Purpura floridana* obtains. From this it is but a step to what occurs in *Buccinum*, *Purpura lapillus* and *Neritina*. In *Fasciolaria* the process is, as far as we know at present, at its culmination.

It does not seem that the breaking down of certain embryos was primarily due to a want of fertilization; in *Urosalpinx* the development proceeds too far and too regularly to allow of this supposition; and the same remark applies to *Purpura floridana*. But we may imagine that in certain forms too little yolk was formed for the number of ova deposited, some change in environment perhaps making it advantageous for the embryos to be retained within the capsules for a longer time than formerly. Some of the ova, then breaking down, were used as nutriment by the survivors, which were thus enabled to persist and develop during their prolonged retention within the capsule. This process might be seized upon by natural selection and increased by it until it became a regular process of the development. Little change would be produced upon the structure, function or activity of the various parts of the reproductive organs of the parent when such a process obtained, but it is easy to see how the

absence of it could by natural selection have modified the relative activity of the germ-producing and yolk-producing portions of the female reproductive organs. An example of this is to be found in *Fulgur*. It is highly probable that the deposition of large numbers of ova was the general rule, but in *Fulgur* the ova are few. Instead of continuing to produce the same number of ova, when subjected to the same conditions as the forms just mentioned, *Fulgur*, or its ancestors, diminished the number of germs produced, while continuing to secrete the same amount of yolk as heretofore. The case is interesting as illustrating how natural selection can accomplish the same end working along two entirely different lines.

It is somewhat difficult, however, to understand how such a curious phenomenon as the death of certain individuals for the benefit of others of the same brood could have been perpetuated by the great law. Instances where the death and use as nutriment of certain germ-cells are produced by the active aggression of others present no difficulty, but this does not seem to obtain in the cases under consideration, the death of the embryo having apparently no relation to attacks of the others. However this may be, another factor steps in, and, in cases where the phenomenon is advanced to any great extent, seems to be invariably present. This is the non-fertilization of the majority of the ova, whereby it is impossible for them to develop to any great extent, and whereby they naturally break down when they have endeavored to segment. We see this in *Neritina*, *Buccinum* and *Purpura lapillus*. In *Fasciolaria*, as stated above, the process reaches its climax, and in this case the sterile nutritive ova do not show the least trace of segmentation, nor do they even show signs of maturation. A nucleus and protoplasm is, however, present, as can be readily seen in sections through embryos which have ingested a number of the sterile ova, in which sections of the ova will of course be present. It seems quite certain that the arrangement in *Fasciolaria* is simply a continuation and specialization of what occurs in other forms, and that we have a fairly complete series of the steps by which the process has been brought about, beginning with such a form as *Urosalpinx* and terminating with *Fasciolaria*.

It is quite conceivable that further specialization yet may

obtain and a case occur in which the nutritive ova do not possess a nucleus and are simply masses of yolk. The nucleus and protoplasm in the sterile eggs of *Fasciolaria* is apparently a waste of substance, and the parent would economize by retaining it. So far as I know, such a case has not yet been described, but it is not improbable that it exists, or, if not, it is likely that it will eventually be produced from a form presenting a condition similar to what we now find in *Fasciolaria.*

II.—THE SEGMENTATION AND FORMATION OF THE GERMINAL LAYERS.

(*a*.) *Descriptive.*

In the earliest stage of *Fulgur* I obtained the polar globule was already formed. It was a comparatively large body (Pl. XXIV, Fig. 3 *pb*) containing yolk-granules apparently, but nevertheless more translucent than the ovum. Owing to its opacity I was unable to perceive any of the karyokinetic changes which precede its formation, nor was I able to detect any of these changes in the cases of the segmentation-spherules for the same cause. A *single* polar globule only is formed in *Fulgur;* I am certain as to this fact, having carefully searched for a sign of a second without success. In *Fasciolaria*, *Purpura*, and other forms in which I have observed their appearance, the polar globules are almost invariably two, and much smaller than in *Fulgur*, agreeing in this respect with what occurs in the majority of forms observed. On no occasion did I observe three or more globules, as Bütschli and Blochmann have described for *Neritina* (9 and 2); it is to be noticed, however, in this case, that two globules only are formed directly from the ovum, the third or remaining globules being formed by division of one or more of these after their extrusion.

I have followed the segmentation in *Fulgur* more completely than in any other forms, and will therefore give first an account of what obtains in that species, indicating only briefly what I have noticed in others.

After the extrusion of the single polar globule the ovum of *Fulgur carica* elongates somewhat, and soon the first segmentation-furrow appears, transverse to the long axis of the ovum, and

dividing it in a plane which passes through the point where the polar globule was extruded (Fig. 4). The ovum is by this furrow divided into two spherules of equal size. They are almost completely separated, and each spherule becomes spherical, but soon they are attracted towards each other again and become flattened along the surface by which they are in contact, so that an ovum at this stage might readily be mistaken for one in which the first division had not yet been completed. This partial fusion of the spherules is succeeded by the second division, whereby the four spherules of the third generation are formed (Fig. 5), the dividing furrow being in a plane at right angles to that of the first division, but like it passing through the point where the polar globule was extruded. If we consider the position of the polar globule to indicate a pole of the egg—and subsequent development shows that it does—then both these furrows are vertical or meridional and divide the ovum into four equal spherules. The same phenomena of complete separation and partial refusion seen in the case of the spherules of the second generation are repeated in those of the third.

At the pole of the egg at which the polar globule occurs, and which may hereafter be termed the formative pole, an aggregation of protoplasm occurs at the point where the four spherules meet in the centre (Fig. 6 *pra*). This protoplasm is derived from the four spherules, and a section through an ovum in this stage (Pl. XXV, Fig. 12) shows that that portion of the protoplasm formed from each spherule remains more or less distinct, so that one might more properly speak of four aggregations of protoplasm, one at the formative extremity of each spherule. In the sections of this stage no nuclei could be distinguished, but a radiating arrangement of the protoplasm could be detected in each aggregation. It is probable that, had the sections been of an ovum at a slightly earlier or slightly later period of this stage, or had reagents more favorable for the observation of karyokinesis been employed, it would have been evident that nuclei were really present, and that this radiating arrangement was merely an indication of approaching division. This division taking place in a plane at right angles to those of the two preceding divisions, and therefore equatorially, four small spherules are separated off containing no yolk-granules, but entirely com-

posed of protoplasm. The fourth generation of spherules thus consists of four small protoplasmic spherules—the micromeres—and four large yolk-containing spherules—the macromeres. The entire amount of material which formed the protoplasmic aggregation preceding this division does not seem to be used up in the formation of the micromeres, since on surface view they are seen to lie in the centre of a protoplasmic area, each micromere being opposite a macromere. The rotation of these first micromeres through an angle of 45°, whereby they come to lie opposite the furrows separating the macromeres, and therefore alternate to these, and which has been described by several authors for other Prosobranchs, I did not observe, and it would appear that it does not obtain in *Fulgur*. It is quite probable that this is due to the fact that the protoplasm from which the spherules of the following generation are formed is already aggregated at the formative pole, and is not, as it seems to be in other instances, aggregated as it is required in the process of division.

The number of macromeres is not increased in subsequent stages, but the protoplasm they originally contained is employed in the formation of additional micromeres; they tend to fuse more and more as development proceeds, until finally, at the period when the organs begin to differentiate, all trace of the furrows originally separating them disappears.

The four micromeres which form the spherules of the fifth generation are derived from the aggregated protoplasm peripheral to the micromeres of the fourth generation (Fig. 7). They lie opposite the furrows which separate the macromeres, and therefore alternate both with the macromeres and with the micromeres of the fourth generation. A section passing somewhat obliquely through the blastodermic area of a later stage (Pl. XXV, Fig. 13) shows the nuclei of the spherules of one generation, but not those of the neighboring spherules. On either side of these latter is seen the aggregated protoplasm (*pra*) not yet separated from the macromeres. To one side of the blastoderm and below it a more or less distinct cavity is to be seen, containing granular matter. It is possible that this may represent the segmentation cavity, though it does not appear to be present in all cases. A very great disproportion in size between the nuclei of the unseparated protoplasm and those of the fourth

generation is noticeable, and in all sections of this and later stages which showed the nuclei of the protoplasmic aggregations their size was very characteristic, being four or five times the size of the nuclei of the spherules, and in addition usually contained numerous nucleoli—nine in one instance—the largest nearly equaling in size the nucleus of a separated spherule.

The four micromeres which form the spherules of the sixth generation are formed by the division of the micromeres of the fourth generation.

The succeeding stages of the segmentation I did not follow in detail, but can state that they result in the increase in the number of micromeres, partly by the division of those already formed, and partly by the separation of new ones from the macromeres. When a considerable number of micromeres have been formed, but while they yet occupy only a very small portion of the area around the formative pole owing to the great size of the macromeres, a phenomenon occurs of which I have found no mention in descriptions of the development of other Mollusca.

On surface view three elongated elevations (Pl. XXIV, Fig. 8, *el*) are seen radiating towards the centre of the blastodermic area, but not extending centrally farther than the edge of the area, and lying rather alternate with the macromeres than opposite them. What the significance of these elevations may be it is not easy to say, but sections through ova at this stage show them to be coincident with the first formation of the *mesoderm*. Such sections, taken from different series, are shown in Pl. XXV, Figs. 14 and 15, and from them it can be seen that the blastoderm, which consisted heretofore of a single layer of cells, now begins to be double, one cell (*me*) lying distinctly beneath the others. This I take to be the first mesoderm cell. Fig. 14 shows on the side of the blastoderm opposite the mesoderm cell an unseparated protoplasm mass (*pra*) with its large nucleus, similar to what has been described above. By tracing a series of sections only three such protoplasmic masses can be seen, the mesoderm cell apparently corresponding to the fourth, and this is supported by what is shown in Fig. 15. This section is somewhat oblique, so that the protoplasmic aggregate opposite the mesoderm cell is not shown, but the mesoderm cell itself (*me*) has an appearance somewhat similar to that of a protoplasmic aggregate, being

large and possessing a large nucleus containing nucleoli. The cell above it also has a large nucleus, but it is well separated off, and cannot represent a protoplasmic aggregate, but probably has recently been formed by division. If this interpretation of the sections be correct, it would seem that the macromere which does not show an elevation on surface view is the one which gives rise to the mesoderm, but what may be the cause of the formation of the elevations on the other macromeres is to me quite uncertain. I think it is safe to conclude that the mesoderm arises by a separation of protoplasm from *one* of the macromeres.

The surface view (Pl. XXIV, Fig. 9) of a somewhat later stage shows a depression (*inv*) situated somewhat eccentrically. External to the blastoderm and opposite each macromere is a small circular elevation (*pra*), which previous stages and sections show to be similar to the protoplasmic aggregates already described. In many cases, beside the depression a comparatively high columnar process can be seen. Sections (Pl. XXV, Figs. 16–19) of this stage show that the mesoderm elements have increased considerably in number, and the depression is formed by an invagination of a portion of the ectoderm. At first I felt inclined to consider this the earliest rudiment of the formation of the shell-gland; but its appearance at so early a stage, while the blastoderm is still limited to a small area at the formative pole of the ovum, and the study of later stages, which showed that the shell-gland did not form until much later, speedily showed the incorrectness of this idea, and convinced me that I had here to do with the invagination of the ectoderm which Blochmann (2) had described for *Neritina*, and with which the depression seen by Sarasin in *Bithynia* (24), and marked *x* in his figures, is probably identical. A series of sections taken transversely across this invagination are shown in Figs. 16–19. In Fig. 16, which represents a section immediately in front of the aperture of the invagination, the ectoderm forms a continuous sheet across the blastodermic area, and below it are seen numerous mesoderm cells; Fig. 17 passes through the mouth of the invagination, and to one side an elevation, formed apparently by a folding of the ectoderm and mesoderm, can be seen; Fig. 18 passes through the centre, and the elevation is seen to form a club-shaped process extending over the cavity towards its opposite

wall, with which in Fig. 19 it becomes continuous—a somewhat similar process growing over to meet it. This club-shaped process apparently in some cases rises up a considerable distance above the blastodermic surface and forms the columnar elevation mentioned above as seen in a surface view. The fate of this ectodermal invagination I was unable to trace, there being unfortunately at this point a lacuna in the series of stages I had preserved for study. In the next stage of which I have specimens no trace of it can be seen, and probably, as Blochmann describes for *Neritina*, it flattens out and disappears. Sarasin does not appear to have recognized the similarity of the depression he observed with that described by Blochmann, although his paper did not appear until a year after Blochmann's. He describes it as a dark spot, which he at first took for a depression, but later it became clearer, a nucleus appeared in it, and it rounded itself off to a spherule. The significance of this ectodermal invagination I do not understand. It seems very strange that an invagination so well marked as it is in *Neritina* and *Fulgur* should disappear and leave no trace of its existence, but so it seems to do. It is strange also that it has not been observed in other forms, but probably this is owing to the few forms in which the processes of segmentation have been followed with the necessary care, and it is highly probable that future observations will show its occurrence in most, if not all, of the Prosobranch Gasteropods.

As stated above, I unfortunately failed to preserve for study by sections the stages immediately following that just described, and accordingly I cannot make any positive statements as to the processes by which the yolk becomes surrounded by the blastomeres, nor as to the formation of the entoderm. In the next stage of which I possess sections the ectoderm forms a continuous sheet around the macromeres, which have now lost all traces of their original separation and form a compact mass. At the formative pole the ectoderm has become exceedingly thin, and its spherules very much flattened, so that in sections it is exceedingly liable to become ruptured to a great extent, allowing the yolk-granules to appear at the surface quite unprotected. The large amount of yolk present in the ova of *Fulgur* proves a great difficulty in the way of preparing perfect sections, on

account of the fragility which it imparts. By preparing some rather thick sections I was able to distinguish easily the thin ectodermal layer at the formative pole. It has been stated that at the stage of the ectodermal invagination protoplasmic aggregates similar to those described in the earlier stages were noticeable. It would seem that in *Fulgur* spherules continue to be budded off from the macromeres for a much longer period than in some other forms; thus, for instance, Blochmann describes only three generations of spherules from the macromeres in *Neritina*. In *Nassa*, according to Bobretzky (4), a greater number are formed; he describes twenty spherules as arising in this manner, and it is possible that more arise in the same way in later stages. Probably the amount of yolk present influences the number of spherules so formed; in other words, the greater the number of spherules required to surround the macromeres, the more frequently are generations formed from the macromeres.

But even after the ectoderm has completely enveloped the macromeres, masses of protoplasm, with large nuclei and many nucleoli, similar in all respects to the protoplasmic aggregates of earlier stages, can be observed in sections (Pl. XXVI, Fig. 23, *pra*), lying sometimes immediately beneath the ectoderm, sometimes imbedded to a greater or less distance in the yolk-granules. Since the ectoderm is already complete, it does not seem probable that these give rise to new ectoderm cells, but it is not unlikely that they may assist in the formation of new mesoderm cells.

In one series of sections through a stage in which the organs had commenced to form I could make out, in the neighborhood of the stomodæal invagination, two of these protoplasmic aggregates, as I have termed them. Apparently a certain amount of entoderm was already formed, but it seemed to me not unlikely that these protoplasmic aggregates might give rise to additional entodermic elements, and that the entoderm spherules already present might have been formed in a similar manner. This would coincide, as far as it goes, with what has been described by Blochmann and others for the entoderm; but any migration of the nuclei of certain of the macromeres from the formative to the nutritive pole, and a subsequent migration of the entoderm elements from the nutritive to the formative pole, as has been

described by the author just mentioned, I could not make out. *Fulgur* is an exceedingly bad subject for the observation of the internal segmentation changes, but it does not seem that the migration of the entoderm cells to the formative pole takes place, nor do Bobretzky's (4) observations show anything of that kind. In *Neritina* the macromeres form that part of the entodermal tract nearest the blastopore, while in *Fulgur* and the forms Bobretzky studied they are pushed away, as it were, from the blastopore by the formation from them of new entoderm cells. This difference is no doubt due to the difference in the amount of yolk present in the various forms.

As regards the blastopore, it evidently occurs at the formative pole, and evidently closes, since in the stages which have been the subjects of the preceding paragraphs the aperture can be detected, the ectoderm forming an unbroken sheet. I did not observe the actual closure of the blastopore and subsequent formation of the stomodæal invagination at that point, but am inclined to believe that that is what takes place.

In other Prosobranchs which came under my observation especial attention was not given to the segmentation. It may be well, however, to indicate briefly the type of segmentation followed by the various forms. *Crepidula* and *Fasciolaria* follow the same plan as *Fulgur*—*i. e.*, the macromeres are equal in size. On the other hand, *Purpura floridana* and *Eupleura* follow the type seen in *Nassa mutabilis*. *Purpura* I followed more fully than any of the others, and the resemblance it bears in its mode of segmentation to *Nassa* is very striking. I was not able to follow in the same egg the formation and subsequent refusion of macromeres which Bobretzky describes, but I obtained stages with five macromeres, for instance, which leave little doubt of its occurrence. The descriptions we possess of the segmentation of *Purpura lapillus* leave very much to be desired; Selenka's (27) description differs so manifestly from what obtains in any other Gasteropod that it must be incorrect.

(*b.*) *Theoretical.*

1. The occurrence of a single polar globule in *Fulgur* seems to me a point of some significance, when the almost invariable presence of two or more in other forms, and the peculiarities of

the ova of *Fulgur*, are taken into account. The idea suggests itself that there is some relation between the polar globules and the amount of yolk present in the ovum, or, to put it more definitely, that the relative amounts of yolk and protoplasm present in the ovum influence the formation of the polar globules. A study of the literature appears to give support to this idea, although it also seems to indicate that there are other factors entering into the question. It will be unnecessary to refer to groups such as the Annelids, in which the polar globules are always produced in a typical manner, but it will be interesting to examine those in which their formation varies, and to observe the concomitant variations in the amount and arrangement of the yolk.

First to consider the Mollusca. They should afford an excellent criterion for the correctness or incorrectness of the theory, since we have the majority of them producing polar globules to the ordinary number, and showing no great disproportion of yolk, *Fulgur* producing only one and possessing a relatively large amount of yolk; and should the Cephalopods, which have a great excess of yolk, prove to have none, the series would be complete. I possess, however, no facts as to the production of polar globules in the Cephalopods, the only papers on the development of forms belonging to that group to which I have access—those of Brooks, Grenacher, and Lankester (in abstract)—making no mention of them. I am strongly inclined to believe that the peculiar segmentation of *Calypiræa* described by Stecker (28) is an error, due to his mistaking the polar globule for the first micromere. It seems not improbable from his description that *Calyptræa* agrees with *Fulgur* in the production of a single polar globule, and with this is associated a comparatively large amount of yolk. Cases in which the macromeres are formed unequal in size, as in *Nassa*, the Pteropods, etc., would seem directly opposed to the hypothesis we are considering, but the peculiarities of segmentation in these forms are not so much dependent upon the presence of a very large proportion of yolk as upon the manner of its distribution. It is not improbable, in fact the very reverse, that the proportion of yolk to protoplasm is much less in the ova of *Nassa*, etc., than in those of *Fulgur*.

As to the Arthropoda, our knowledge of the processes of maturation and fecundation of the ovum is as yet very fragmentary, and it is only very recently that any forms of the group have been definitely ascertained to produce polar globules. In certain cases where one would expect them to be formed, as in *Lucifer* (Brooks) and *Penæus*, they have not been described; but, on the other hand, in a small but increasing number of forms which possess a comparatively small amount of yolk, structures which have no doubt correctly been homologized with polar globules have been found. Thus, to give a list of cases which I have been able to find, both Kennel (44) and Sedgwick (54) have described two polar globules in *Peripatus*; Grobben (35) observed two polar globules form in *Cetochilus*; the same author (34) has described in *Moina* a body, lying at the animal pole of the egg, but not separated from it, which he considers a polar globule, but has no facts to give concerning its origin, which takes place within the ovary; in a foot-note Grobben refers to an observation by Leydig as to the occurrence of bodies quite similar to polar globules which lie at one pole outside the egg-membrane in *Daphnia longispina*; Henneguy (40) has observed two globules in the egg of *Asellus aquaticus*, one of which he saw detach itself from the yolk, and states that in some ova there were four globules, which were, however, smaller than those in the ova in which there were only two, and believes that the greater number is due to the original two globules having divided; and lastly Hoek (43) describes, in the earliest stage of *Balanus* which he observed, a single protoplasmic body lying between the yolk and the egg-membrane, which he considers a polar globule. In this last case there is present apparently a considerable amount of nutritive yolk, and it is interesting to note that if the structure really is a polar globule, only one is formed. In the Arthropoda the presence of a large quantity of yolk is the rule, its absence the exception; and this fact, on the supposition that a large quantity of yolk influences the production of polar globules, explains their absence in the majority of the forms which have been observed. The only cases I have found in which polar globules have been described associated with a relatively large quantity of yolk are those of *Musca vomitoria* and *Chironomus*, the development of which has been studied by Weismann (57).

That the problematical bodies he describes as pole-cells really are of the nature of polar globules seems improbable, and Balbiani (32) very recently expressly draws a distinction between the two structures in *Chironomus*. According to Weismann four of these pole-cells are formed after the aggregation of the blastema at the surface of the yolk. They afterwards increase in number, and in the later stages of development they again unite with the blastoderm.

When we consider how very recent is the discovery of polar globules in any of the Arthropods, it seems probable that further investigation will show their presence in all forms in which the yolk is not in very great excess. It must be noticed, however, that departures from the normal occur in the formation of the polar globules in certain forms, such as *Moina*, which cannot be referred to a superabundance of yolk, for we find instances—*e. g.*, *Asellus* and many Mollusca—in which, although a relatively much greater quantity of yolk is present, normal globules are produced. It seems certain that, in addition to the yolk, other factors influence the formation of polar globules, but what these may be can only be determined when further data are obtained. Even, however, taking into consideration such cases as *Moina*, it seems to me that the evidence to be obtained from the Arthropods points to a correlation between the yolk and the polar globules.

In the Chordata the state of affairs is very interesting. In the Ascidians, the ova of which as a rule possess little nutritive yolk, the formation of polar globules is the rule. *Amaræcium*, however, has a relatively large amount of yolk, and it would be exceedingly interesting to know certainly of the presence or absence of polar globules in this form. In the researches of Maurice and Schulgin (49) upon its development no mention is made of their formation, and, since stages in which they could have been seen, if present, were under observation, it seems likely that they were absent, although the alternative that they may have been overlooked must be considered, the presence of the "test-cells" rendering such an occurrence by no means improbable. *Amphioxus* has, according to Hatschek (37), only one polar globule—a fact rather in opposition to the idea I have advanced, since in the ova of this form the yolk is exceedingly small, allowing of a total and regular segmentation. Whether

future observations will reveal the presence of a normal number of polar globules remains to be seen.

In the Vertebrata with an unequal segmentation we find a very decided modification of the typical arrangement. Kupffer and Benecke (46) have described in *Petromyzon* the formation of one polar globule before and another after (?) fertilization, and Scott (53) confirms the presence of the second one; it seems doubtful if the identification of the first-formed structure as a polar globule is absolutely correct. Salensky describes (52) in *Acipenser* a veil-like body extruded from the ovum, consisting of a transparent, almost homogeneous substance, which he compares to the veil-like body described by Hertwig (41) for the Amphibian ovum. These cases accord very satisfactorily with the hypothesis suggested.

In meroblastic vertebrate ova I have found only three cases in which polar globules or structures supposed to correspond to them have been described. Oellacher (51) has stated his belief that there is a complete extrusion of the germinal vesicle in the trout and the chick; from our present knowledge of the phenomena of fecundation we must consider that his statements are not altogether correct; but, even granting that something is extruded from the ovum, it is to be noticed that the process is much modified from that which obtains in the formation of typical polar globules. Balfour (33) has observed phenomena in the ova of Elasmobranchs which have led him to believe that the membrane of the germinal vesicle *is* extruded; but he did not observe the actual extrusion of any portion of it, and his conclusions are therefore not perfectly reliable, as he himself has admitted. Nevertheless, here too, granting that the membrane of the germinal vesicle is extruded, there is a wide departure from the normal formation of polar globules. Lastly, Hoffmann (42), in certain Teleosts, describes the germ-nucleus as approaching the surface of the ovum and forming a "Richtungs-spindel," the peripheral portion of which is extruded from the egg, forming a polar globule, while the central portion forms the female pronucleus. Hoffmann's description is so circumstantial that one cannot but believe that in the forms observed by him a polar globule is produced, but only *one*, and not two, as in the typical cases. In the Reptilia no structures which can be homologized with polar globules have been observed.

From the researches of Van Beneden (56) on the development of the Mammalia, it is certain that in the forms which have been studied two or three polar globules are formed; and this is exceedingly important as bearing on the theory I have advanced, since concomitantly with the almost entire absence of yolk we find the polar globules formed in a manner more nearly approaching the typical method than in any of the Vertebrata above *Amphioxus.* That the Mammalian ovum has descended, so to speak, from ova with a very large supply of nutritive yolk, there cannot be the slightest doubt; peculiarities in the early stages of their development, and the phylogeny of the group, both point to the same conclusion, and it is interesting to observe that their ova have not retained the rudimentary formation of polar globules which was no doubt characteristic for their ancestors, but have reverted, so to speak, to a more primitive condition, this reversion being dependent on the loss of the voluminous yolk.

It seems to me that the facts detailed in the preceding pages, fragmentary though they be, point very strongly to the correctness of the theory that *the relative amount of yolk present in the ovum influences the formation of the polar globules.* In cases where there is an excess of formative material, the globules are present, as a rule, in their typical number and form; but when there is an excess of nutritive material they are either entirely absent or are represented in a very much modified condition. As stated above, however, there is little doubt but that other factors influence their production, such as heredity, perhaps, and to their influence are to be ascribed certain abnormalities which cannot now be explained. Until these other factors have been determined, we cannot form any certain hypothesis as to the significance of the polar globules.

2. Rabl (22) has shown conclusively that the apparently very dissimilar modes of segmentation to be observed in the Gasteropoda are merely modifications of one and the same type, and depend on the arrangement of the food-yolk in the different ova. Fol (12) previously had stated his belief in the similarity of the segmentation in all the Cephalophores, and was inclined to include in the same category that of the Lamellibranchs. He offered, however, no explanation of the manner in which the various modifications had been brought about. It was Rabl who first

did this for the Cephalophores, and Brooks (8), from his researches on the oyster, was able to show how the Lamellibranch segmentation could be referred to a modification of that of the Gasteropods. Recently Ziegler (31) has dissented from this view on the ground that in the Lamellibranchs the only spherule in which unequal division obtains is that in which the food-yolk is aggregated. It does not seem that this objection is sufficiently weighty to overthrow Brooks's conclusions, especially since we should expect exactly what occurs from our knowledge of the influences which modify segmentation. We know that unequal segmentation depends on the relative amount of yolk which the ovum contains, and of course the same influence will act in the spherules. If, then, the greater quantity of the yolk-material is aggregated in a single spherule, the others being composed almost entirely of protoplasm, we should expect to find in the latter equal division, and in the former unequal. Brooks has derived the segmentation of the Lamellibranchs from such a type as is shown in *Nassa*, *Urosalpinx*, etc.; this type, however, has undoubtedly been derived from that in which the four macromeres are equal and contain an equal amount of yolk, which appears to be most typical for the Gasteropods, occurring apparently in all the Pulmonates, the majority of the Prosobranchs, in the Heteropods, and apparently also may be looked for in the gymnosomatous Pteropods. I would prefer to derive the Lamellibranch segmentation originally from one in which the macromeres were all equal, but they must certainly have passed through a stage similar to that represented by *Nassa*.

Several authors have pointed out the similarity which exists between the early stages of segmentation in the Mollusca and those of the Turbellarians, Planarians and Hirudinea, and it seems to me that this similarity is of great importance. I believe that it is not an accidental similarity, independently acquired, but is a similarity of phylogenetic origin. The objections which have been urged, founded upon the different origin of the mesoderm elements in the various groups, will be discussed hereafter; it will be more suitable here to discuss the origin of the segmentation of the Annelids and other groups, which bear, through their trochophore larva, a much closer relation to the Mollusca than the groups just mentioned.

As regards the Annelida (excluding for the present the Hirudinea), we do not possess sufficient data as to the segmentation of the Oligochæta to make any definite statements regarding them. Kleinenberg's description (45) is very imperfect, no detailed account being given of the origin of the various spherules. It seems not improbable, however, that there are present four macromeres, two large and two small, from which four micromeres are formed; such an arrangement may be deduced from his description and figure. It will not be necessary to review the numerous papers on the development of the Polychæta, but a reference to Wilson's paper (58), in which special attention is paid to the modifications of segmentation in several forms, will be sufficient. In all the Polychæta he investigated the amount of yolk was not particularly great, and a perfect correspondence with the Mollusca can hardly be expected. In *Clymenella torquata* four macromeres, somewhat unequal in size, develop, and from these four micromeres are formed, not quite simultaneously, but the intervals are very small; both micromeres and macromeres then divide. The resemblance of this segmentation to that of the Mollusca is quite evident, and that of *Arenicola cristata* is very similar, the micromeres, however, being larger in proportion to the macromeres—an arrangement carried still farther in *Chætopterus pergamentaceus*, where they are almost equal, if not quite so. It is interesting to notice that, according to Hatschek (39), in *Eupomatus uncinatus* (originally *Serpula uncinata*) the four spherules at the formative pole of the egg in the eight-spheruled stage are slightly smaller than those at the nutritive pole, and this, when the quantity of yolk is so small, and apparently so equally distributed, that one would expect a perfect equality. Can this be explained as an arrangement inherited, so to speak, from an ovum with yolk-containing macromeres? It seems to me exceedingly probable that the segmentation of the Annelida is to be derived from a type resembling very closely that of the Mollusca, modifications of the original type, however, as in the Lamellibranchs, having been brought about by the loss of nutritive yolk, and by abbreviation of the development.

Our knowledge of the segmentation processes in the Polyzoa, Rotifera and Brachiopoda is so slight that nothing can be said

concerning them. Among the Gephyrea, however, Spengel's observations on *Bonellia* (55) show that its segmentation is very like that of the Mollusca.

It seems very probable, as before stated, that these similarities are of phylogenetic significance. If the doctrine that the ontogeny of any form is a recapitulation of its phylogeny be accepted, making allowance, of course, for the action of natural selection in the various forms, it follows that even in the segmentation indications of phylogeny may be looked for. The ovum is necessarily an homologous structure in the various groups, and all Metazoa pass through stages the result of which is the formation of a many-spheruled organism; all those forms whose common lines of descent include the stages of spherule-formation should show a greater or less similarity in their modes of segmentation. This similarity will be obscured by secondary adaptations, so much so as to be almost or quite hidden, but it should show itself to some extent in some of the groups, and this it does in those which have just been discussed. The Ctenophores develop by an unequal segmentation, and it is probable that from such ova, by the assumption of a greater quantity of nutritive material and by a tendency for it to collect at one pole of the ovum, the mode of spherule-formation which has been indicated as typical for the Mollusca has been brought about. This accords very well with the origin for the Turbellarians proposed by Lang (47), who refers these forms to a Ctenophore-like ancestor. The Nemerteans have a more regular segmentation, but this can be explained as a secondary arrangement produced by loss of yolk, and the slight inequality of spherules which the Nemerteans do show indicates a relationship between their segmentation and that of the Turbellarians. The arrangements which obtain in the Trematodes and Cestodes need not be discussed here, nor those of Nematodes, so little is accurately known concerning their spherule-formation, and the conditions under which they, as a rule live, are so peculiar. The Annelida most probably have been derived from forms represented by the Turbellarians, and we find in the Hirudinea a similarity to the Turbellarian mode of spherule-formation, and, as I have briefly attempted to show, the Oligochætous and Polychætous segmentation has been derived from the same type. Lastly, the Mollusca

show through their larvae that they and the Annelida have a common line of descent as far as the formation of the trochosphere stage, and their segmentation agrees with the common type which we have traced all through these various groups. It remains to be seen whether the Polyzoan, Rotiferan and Brachiopodan segmentation can be referred to the same type.

To state definitely the idea which I have attempted to develop in the preceding pages, *the mode of segmentation of the Platyhelminths, Annelida, Mollusca and Molluscoidea can be referred to a common type, indicating that the ovum (so to speak) in all these groups has been derived from an ovum possessing a considerable amount of nutritive yolk aggregated more or less completely at one pole.*

That the Vertebrata may be included in the above list of groups seems not improbable, indications of the original type being shown in the unequal segmentation seen in some forms—*e. g.*, Amphibians—but I do not intend to discuss this here.

It follows from what has been said that *the regular and equal segmentation which occurs in certain forms in several of these groups cannot be considered the original mode, but has been secondarily brought about by the loss of a food-yolk originally present.* This view is in direct opposition to that of embryologists who have studied these groups. As Rabl (21) has pointed out, the ovum begins its struggle for existence the moment it separates from the mother, and it is apparent that there would be a tendency for the ovum to acquire a store of nutritive material in order that it might be absolved for a time from the necessity of struggling for a supply of nutrition, without which its development could not proceed to any extent. Those forms which secondarily have been withdrawn from the struggle, by being retained within the body of the mother or in a special brood-cavity, would not require so large a supply of nutritive material, and the food-yolk in these forms would gradually be lost. Among the Prosobranchs *Paludina* is viviparous, and its ova are characterized by possessing a very small amount of food-yolk, and therefore by an equal segmentation; and among the Annelida some, if not all, of the Serpulidæ, which show an equal or nearly equal segmentation, are retained in a special brood-pouch. Another point, however, has to be taken into consideration—

namely, that concomitantly with the absence of much food-yolk there is a hastening of the segmentation processes, the specialization of the germinal layers, the development of locomotor structures, and the formation of a digestive tract and gastrula-mouth; so that the tendency to lose a supply of nutritive material might be advantageous to a form, by allowing it to hasten over the stages during which it is at the greatest disadvantage in the acquisition of nutritive material. The absence of yolk in *Amphioxus* and the Echinoderms, etc., may perhaps be explained in this way. Moreover, the very fact that a large number of forms, by having a supply of nutrition already stored up when set free, are thus withdrawn from the struggle for food during the early stages of development, would render it all the more easy for yolkless forms to obtain nutrition during their early stages.

It seems to me that the occurrence of what may be termed an irregular segmentation in the Lamellibranchs and the Annelida, accompanied by the presence of a quantity of yolk apparently not sufficient to produce any departure from a regular process, is in itself an indication of the derivation of these forms from ancestors whose ova possessed a comparatively large amount of food yolk.

3. The early formation of the mesoderm is quite in accordance with the more recent researches in the Mollusca, as is also its origin from a single cell belonging to the nutritive pole of the egg. Blochmann's beautiful researches on *Neritina* (2) show one of the macromeres giving rise to a protoplasmic cell, which divides and so forms the mesoderm; and Bobretzky's results seem to indicate the same arrangement in *Nassa*. According to Wolfson (30) the mesoderm arises in *Lymnæus* during the blastula-stage from the macromeres. Rabl shows that in the Pulmonates (22) and Lamellibranchs (21) the mesoderm arises by the division of a spherule at the nutritive pole of the egg, and Hatschek describes (15) a similar origin for it in *Teredo*. In these last cases the primitive mesoderm cells are arranged bilaterally, and are situated immediately behind the blastopore. The apparent differences between the results of Rabl and Hatschek and those of the other authors mentioned above can be readily explained. The presence of the large quantity of yolk in *Fulgur*,

Neritina, *Nassa*, etc., requires the separation of a protoplasmic cell which corresponds to the spherule in *Teredo* and *Planorbis*, which by division gives rise to the bilaterally arranged mesoderm cells. The relation of this cell to the blastopore is also equivalent to the original mesoderm cell in *Teredo*, etc. It lies immediately below the margin of the ectodermal cells, which margin corresponds to the margin of the blastopore in the forms with a small quantity of yolk, the formation of the mesoderm in both yolk-containing and comparatively yolkless eggs taking place at very nearly the same time as regards the number of segmentation spherules, which brings it about that in the former class of eggs it is formed while the ectodermal cells are still confined to the formative pole of the egg, and the margins of the blastopore are still very widely separated.

The researches of Bütschli on *Paludina* (9), and Brooks on the Pulmonates (6), in both of which the mesoderm is described as arising from the neighborhood of the blastopore, probably indicate an arrangement similar to what has been described above; their observations having been made without the aid of sections, the real relationships of the mesoderm may readily have been overlooked. The same remarks apply to Blochmann's results in *Aplysia* (3), in which form no sign of mesoderm was to be seen until a comparatively late stage, it being first clearly discernible at the time when the œsophageal invagination had formed, and then was arranged in such a manner that it appeared to have arisen from the sides of the invagination, which, it must be noticed, formed, as in *Fulgur*, at the point where the blastopore closed.

There are observations, however, which cannot be reconciled with those mentioned above, and which seem to indicate that the mesoderm may arise in part in a somewhat different manner. My study of *Fulgur* shows that while the mesoderm arises by the separation of a cell in the neighborhood of the blastopore, yet in later stages it receives additions in the shape of cells separating irregularly from the macromeres. Certain observations seem to point to the occurrence of a similar secondary formation of mesoderm in other forms. Thus Fol expressly states that in the Pteropods (11) and Heteropods (12) the middle layer arises as local separations from the ectoderm; and Sarasin,

from a study of *Bithynia* (24), came to the conclusion that although the mesoderm forms in part in the neighborhood of the blastopore, yet it is impossible to speak of a special mesoderm as distinct from the ectoderm.

Hatschek (15) lays great stress upon the early appearance of a bilateral symmetry in *Teredo*, and concludes that all the Bilateria come from an ovum in which bilateral symmetry is potentially present. I quote his own words: "Und ebenso wie an der Keimblase wird auch schon an den Furchungstadien und auch an der Eizelle eine bestimmt rechte und linke Hälfte, ein bilateral Bau bei allen Bilaterien vorhanden sein."[1] It seems to me that Hatschek has erred in making a generalization from a special and what may be called an abnormal case. As I have indicated above, there is reason to believe that the peculiarities of the Lamellibranch segmentation are due to its being derived from such a type as is seen in the Gasteropods. Now, in this the bilateral symmetry is first indicated when the mesoderm is formed; previously to this one axis only can be distinguished, that connecting the formative and nutritive poles, and around this axis the spherules are arranged radially, no special transverse axis being discernible. In *Fulgur* and all forms which show the typical Gasteropod segmentation, a bilateral symmetry is present neither in the segmentation-stages nor in the egg-cell; it first makes its appearance when the three germ-layers become differentiated. Accordingly the bilaterality in the segmentation-stages of *Teredo* is entirely a secondary affair.

But was the mesoderm itself originally a bilateral structure? This is a question which presents great difficulties, but Metschnikoff's recent researches show that in the Ctenophores, which probably represent the ancestral forms of the Bilateria, the mesoderm is a bilateral structure. If we assume the Gastræa theory to hold in so far as the Bilateria, including therein the Ctenophores, are concerned, thus omitting the moot-point as to its applicability to the typical Cœlenterata, in the line of descent of the Bilateria we would have first a uniaxial Blastæa, from which would result by invagination a uniaxial Gastræa. The

[1] Brooks had previously shown that the oyster-embryo is bilaterally symmetrical from the earliest stages of segmentation, and that the egg itself must be held to be bilateral.

mesoderm probably appeared in the phylogenetic history of the Bilateria at this stage, and, appearing bilaterally, transformed the uniaxial into a biaxial Gastræa.

The mesoderm, when it first arises in the Planarians, is, however, radially arranged. It appears in these forms very early, according to Lang's description (48), being formed from the four cells of the fifth generation—*i. e.*, from the second group of micromeres which is segmented from the macromeres—and these, since the segmentation of the Planarians is on the same type as that of the Gasteropoda, are radial. This arrangement, however, is secondary, and depends upon the law formulated by Hatschek (15) in the following words: "Bildungen, die einen Entwicklungstypus tief beeinflussen, sehen wir immer ontogenetisch früher auftreten, als es der phylogenetischen Zeitfolge entsprechen würde." The mesoderm in most of the Bilateria, instead of forming ontogenetically at the same stage as that in which it appeared phylogenetically, forms at a much earlier stage—during the segmentation—and in the Planarians this hastening of its appearance is carried to such an extent that it forms the fifth generation of segmentation spherules. In this case, instead of retaining its original bilateral arrangement, it conforms to the radial symmetry which still dominates—a point which indicates how firmly the radial symmetry is implanted in the early stages of segmentation.

From what has been said, it is evident that the differences in the formation of the mesoderm in different groups cannot afford any basis for the support of objections to the existence of a phylogenetic significance in the similarity of the segmentation in these groups. These differences are secondary, and depend simply upon the extent to which the hastening of the formation of the mesoderm has been carried. Had the mesoderm retained its original time of formation, and first appeared in the gastrula stage, it can be conceived that the segmentation of the Gasteropods, Planarians, etc., would have been identical, making allowances, of course, for the modifications produced by the amount of yolk present in individual cases.

III.—THE VELUM.

Before passing on to describe some of the embryonic organs in detail, it will be well to make a few remarks concerning their general development. The shell-area (Pl. XXIV, Fig. 10 *sh*) appears early, as is usual, and indicates the posterior extremity of the embryo, so that it becomes easy to discern the anterior and posterior extremities, and the right and left sides. The organs make their appearance near the shell-area, and consequently nearer the posterior than the anterior extremity of the embryo; but as the shell develops they gradually pass forwards (cf. Figs. 10 and 11) until they come to lie at or near the anterior extremity, and at the time when the embryo passes into what may be termed the larval stage they compose the anterior portion of the embryo, the large yolk-mass, below which they formerly were situated, being entirely covered and contained by the larval shell. In the earlier stages the embryo is somewhat pyriform in shape, the small end being covered by the rudimentary shell, the ventral portion of the middle region being occupied by the developing organs, such as the mouth, velum, foot, and primitive excretory organs, while the anterior or larger end consists of the yolk-mass enclosed in a very thin layer of ectoderm. As the shell grows and the organs migrate forwards, this pyriform shape disappears and the embryo again becomes oval. In consequence of this posterior position of the organs at their formation, and since they lie ventral to the large yolk-mass for so long a time, little can be made out concerning them by studying the embryo *in toto;* it is necessary to have recourse to sections. For the same reason the head-vesicle, so characteristic in general of the Gasteropods, does not show distinctly until, in the process of forward migration, it reaches the anterior extremity.

The velum (Fig. 10 *V*) arises in *Fulgur* as a narrow, ridge-like elevation extending on either side from behind forwards and inwards towards the point at which the mouth-invagination (*M*) occurs. Each ridge is formed by a folding of the ectoderm, and is not a thickening. It is at first almost straight, but soon, near the mouth-invagination, makes an abrupt curve outwards again, not meeting its fellow of the other side in front of the mouth at this stage; nor is there a meeting upon the dorsal

surface, the structure extending up on the sides of the embryo only a very short distance. Behind it are the so-called primitive urinary bodies, and in front of these, lying between the two velar folds, are two eminences (*F*), the origin of the foot, which is thus in its formation a paired organ. Still later the anterior portion of the velar fold curves inwards again (Fig. 11), so that the anterior extremities of the ridges of opposite sides approach one another in front of the mouth, and eventually meet at that point, each half of the now single velar ridge being somewhat *s*-shaped. Dorsally the two extremities are still ununited, and remain so during the whole larval life. The ridges or folds are ciliated and form a pre-oral band of cilia. Whether this band is double or not I cannot state positively, but am inclined to believe that, in its earlier stages, at any rate, it is. At the time when the head-vesicle has reached the anterior extremity of the yolk-mass, and begins to project beyond it, the velar folds have increased considerably in size, forming two double-walled laminæ projecting out on either side, the embryo being, as it were, in the velar region pulled out laterally. This pulling out continues in later stages until finally the large velar areas are formed on either side of the head-vesicle, each being semicircular in shape, with the anterior surface flattened and the border furnished with the powerful velar cilia. Mesoderm cells make their way at an early stage into the space enclosed by the velar fold, and soon begin to elongate and develop into the muscular fibrillæ of the velar area, forming in later stages a network of muscle-fibres, and producing contractions of the velum. In advanced embryos, and in the larval veligers of several marine Prosobranchs, I observed, in addition to the pre-oral band of cilia which runs along the margin of the velar area, certain other well-defined ciliated bands and areas which are of considerable importance. I have already given an abstract in the University Circulars (20) of these observations, but it will be necessary to discuss them here more in detail.

In a veliger obtained by means of the surface net (Pl. XXVI, Fig. 25), whose relationships I have not been able to identify precisely, it was easy to distinguish the characters of the velar ciliated bands. The velum (*V*) is large and each half is bilobed. Around the margins of the lobes are the strong cilia of the pre-

oral band (*Pro*), which in a ventral view can readily be traced across the body of the larva anteriorly to the mouth. Upon the under surface of the velar lobes—the foot of the larva being directed towards the observer—a band of smaller cilia (*Poo*) can be seen not very remote from the pre-oral band. This band can also be traced across the body of the larva to meet with its fellow of the opposite half of the velum, but passes, in contrast to the pre-oral band, *behind* the mouth, and may therefore be termed the *post-oral band.* Between these two well-marked bands a small area is enclosed which is lined with very delicate cilia, still smaller than those of the post-oral band, and which are continuous with the similar cilia lining the œsophagus. These, to adopt Hatschek's term (36), may be called the *ad-oral cilia.* The diagrams shown in Pl. XXVI, Figs. 22 and 24, which are modified from unpublished drawings kindly placed at my disposal by Dr. W. K. Brooks, will show clearly the relations of these various ciliated areas. Fig. 21 is a diagrammatic ventral view of a Prosobranch Gasteropod, in which *Pro* denotes the strong pre-oral, *Poo* the weaker post-oral cilia, and *Ad* the ad-oral region with its cilia; while Fig. 24 represents a diagrammatic section through half of one of the lobes of the velum, the lettering having the same significance as in Fig. 22.

I first accurately perceived these various ciliated bands and their true relations in the larvæ of *Crepidula fornicata*, and was afterwards able to confirm my observations on that form by a study of the embryos and larvæ of *Crepidula convexa*, *Fulgur carica*, *Fasciolaria tulipa*,[1] and of a second veliger obtained in the surface net, perhaps that of *Neverita.* It seems highly probable that the same arrangement of the velar cilia will, by future observations, be found to be characteristic of all Prosobranch veligers.

The true relations of the pre-oral band have long been known, and the existence of the post-oral band has also been described, but its true relations have not hitherto been clearly defined in the Prosobranchs. In other Molluscan groups it has been recognized to be a post-oral band. In the Opisthobranchs it was first indicated by Lankester (17), who, in his Fig. 17, Pl. 8 of the larva

[1] In the abstract in the University Circulars this form was erroneously referred to the genus *Neptunea.*

of *Pleurobranchidium*, represented a band of cilia lying in front of the root of the foot and behind the mouth and passing dorsally on either side to meet the pre-oral cilia at an acute angle; and in his Fig. 8, Pl. 10 of the veliger of *Polycera* the post-oral band is represented passing around the velar area, but is not shown to pass behind the mouth. In the text no notice is taken of the post-oral cilia, however, and it was Haddon (14) who first demonstrated accurately its relations in the Opisthobranchs, as well as the presence of the ad-oral cilia. Fol, in the Pteropods (11) and Heteropods (12), recognized a band of cilia behind the pre-oral locomotor band, but did not observe that it passed behind the mouth. In his paper on the development of the Heteropods he says: "Au-dessous des cils moteurs se trouve la même zone de petits cils-nourriciers que j'ai decrits pour les Ptéropodes (p. 136) et dont la signification physiologique est d'amener les particules nutritives à la bouche." For the Lamellibranchs Hatschek (15) demonstrated in *Teredo* the existence and relations of all three ciliated bands—*i. e.*, the pre-oral, post-oral and ad-oral. Brooks (7), in a description of the Gasteropod velum—the Prosobranch *Astyris* serving as the type—writes as follows: "The mouth is not within the circlet of large locomotive cilia, but immediately behind it, and a ring of smaller cilia passes from the anterior margin of the mouth entirely around the velum, on its lower surface, and therefore outside the circlet of locomotive cilia. This second circlet seems adapted to convey food to the mouth, but there are no direct observations on this point." The use of the term "anterior" in this description is rather ambiguous, and would lead one to infer that the nutritive band was pre-oral as well as the locomotor. Dr. Brooks informs me, and drawings which he has placed at my disposal show clearly, that he really observed the post-oral position of the band of nutritive cilia, and that the term "anterior" refers to a ventral, and not a dorsal position. Brooks is therefore the first who has demonstrated the existence of a post-oral band of cilia in the Mollusca.

The researches of Fol upon the Pteropods (11), Rabl on the Pulmonates (22), Hatschek on the Lamellibranchs (15), and Bütschli on the Prosobranchs (9), show that in certain forms, at any rate, in all these groups, the cilia of the pre-oral band are at one time arranged in two rows, and my own observations on the

Prosobranchs were to the same effect. It is probable that it is the typical arrangement in all these groups. The post-oral band consists of a single row of ciliated cells. It seems safe to conclude that *in the Lamellibranchs, Pteropods, Heteropods, Opisthobranchs and Prosobranchs the ciliation of the velum consists of a pre-oral double band with powerful cilia, a post-oral single band with weaker cilia, and between these an ad-oral area covered with very delicate cilia, which are continuous at the mouth with those lining the œsophagus.* It is also highly probable that the Pulmonate larva has descended from a veliger with a similar velar ciliation, but the typical arrangement has been lost to a great extent by the reduction which has supervened in the specialization of the velum in this group.

IV.—THE EXCRETORY ORGANS.

Fulgur, like the majority of Prosobranchs which have been investigated, does not exhibit the formation of a "head-kidney," but the primitive excretory system is formed by a clump of ectoderm cells similar to those Bobretzky (4) has described for *Nassa*. These primitive excretory cells make their appearance very early, and at the time when the velar ridge first becomes apparent they also become visible as an irregular elongated elevation running parallel and posterior to the velar fold (Pl. XXIV, Figs. 10 and 11). In transverse section, at an early stage, each elevation has a rosette-like appearance, the cells composing it being heaped up above the general surface of the embryo and loosely aggregated together, but in other respects closely resembling the adjoining ectoderm cells. Soon, however, their appearance changes; vacuoles begin to collect in their interior, which increase in size, fuse together, and eventually displace the protoplasm of the cell almost entirely, the nucleus being forced away to one end of the cell close up against the cell-wall. As the embryo grows the secretory cells retain their original position relative to the velar fold, though at the larval stage they have increased considerably in number and extent. At this stage they form a mass broad below, where they extend down upon the sides of the foot, and tapering above as they pass towards the dorsal region of the body, but lying some distance behind the margin

of the velum, at the point where its posterior wall merges into the body-wall.

In *Fasciolaria*, however, the primitive excretory organ enters into much closer relations to the velum. I have not been able to study its development in this form, but in more advanced stages it forms a large and very apparent mass upon either side of the head-vesicle of the embryo, the velum being quite small in proportion. As the velum increases in size, however, the excretory organ comes to lie about the middle of its posterior surface, forming a hemispherical projection of considerable size, appearing as if composed of radiating hexagonal columnar crystals. A section through the velum at this point (Pl. XXVI, Fig. 26) shows well its structure. It is seen to consist of large columnar ectodermal cells (*Ex*), hexagonal on cross-section from mutual pressure, which are completely filled with a dense refractive albuminous substance. The cell-walls are thick, and can be seen in some places to be separated from the cell-contents —a condition probably due to a contraction of the latter from the action of the hardening and preserving reagents. In each cell a nucleus (*N*) can be seen usually lying towards the outer extremity of the cell and pressed closely against the cell-wall, but in some cases enclosed within the albuminous cell-contents. According to Bobretzky (4), in *Fusus* the exceedingly thin ectoderm passes unbroken beneath these excretory cells. I was able to detect no such arrangement in *Fasciolaria*, but at the edges of sections, of one of which Fig. 26 represents a part, the excretory cells could be seen to pass directly into the normal ectoderm cells and to be continuous with them. None of my sections of *Fulgur* allowed of any definite conclusion as to this point.

The question as to the comparative significance of these peculiar excretory cells of the Prosobranchs has already been discussed by several authors. A brief *résumé* of their opinions will not be out of place. Omitting the older observers, Bütschli (9) may be mentioned first. He observed in *Lymnæus* and *Planorbis*, on each side at the posterior bend of the velum, and immediately anterior to the "head-kidney," three large cells filled with yellow granules. In *Paludina* there are no excretory cells present, but a "head-kidney" does exist; the cells which

bear the pre-oral cilia, however, are richly provided with yellowish granules. Fol (13) describes in aquatic Pulmonates, below the cells of the pre-oral band, a row of vacuolated cells, at the posterior extremity of which the "head-kidneys" form. He suggests that there may be some kind of relation between these two excretory organs—a serial homology, as it were. It may be mentioned that Fol describes the pre-oral band of cells of the Pteropods (11) as containing refractive granules. Rabl (22) describes the pre-oral ciliated cells of the velum in the Pulmonates (*Planorbis*) as containing yellowish granules, and gives it as his opinion that they have nothing to do with the "head-kidneys." Sarasin (24) describes a very close relationship between the excretory cells and the pre-oral ciliated cells in *Bithynia*, their connection being so intimate that he describes them together as the "ansæ." He observed what he took to be openings upon these cells, which he compared to the opening of the primitive "head-kidney" of other forms, and which lead into a cavity in the interior of the "ansæ." If these excretory cells were not united to the velum, he would have no hesitation in calling them a "kidney." The conclusions he draws from his observations may be quoted. He says: "Nach den Erfahrungen von Bobretzky, Bütschli und mir liegt auf jeder Seite der Prosobranchier-Embryonen ein Häufchen grosser ectodermzellen, das bei *Paludina* und *Bithynia* mit Wimperöffnung nach aussen mündet. Nach Bütschli und Fol finden sich dieselben bei *Planorbis.* Ist dies richtig, so haben die Süsswasser-pulmonaten zwei Organpaare, die als Urniere zu deuten sind, ein vorderes und ein hinteres Paar; letzteres mit der interessanten grossen Secretionszelle. Hat Rabl recht, dass die von Bütschli zuerst gefundenen grossen Zellen bei *Planorbis* und *Lymnæus* zum Velum gehören, so sind wahrscheinlich die von Bütschli und mir bei *Paludina* und *Bithynia* gefundenen Organe den hinteren Urnieren der Süsswasser-pulmonaten Homolog. Dies wird nun noch des weiteren zu untersuchen sein."

The true arrangement in *Bithynia*, however, is probably that which Rabl describes (23). According to his observations the velum is closed dorsally, and its lateral and dorsal portions consist of large transparent cells filled with a refractive fluid. These he compares to the excretory cells of the Prosobranchs. "Head-

kidneys" are also present, so that the arrangement is identical with what occurs in the aquatic Pulmonates.

In the first place, it is interesting to notice that it is the two genera of Prosobranchs which most nearly approach the aquatic Pulmonates in their habits of life, which show the presence of "head-kidneys," while on the other hand these structures have been described in no marine Prosobranchs.

In the second place, it would seem that the arrangements in *Paludina* and *Bithynia* give a key to the relation of the primitive kidneys and the excretory cells. In both the primitive kidneys are present; in *Paludina* the excretory cells are represented by the cells of the pre-oral ciliated band in its entirety; in *Bithynia* by the lateral and dorsal cells of the pre-oral ciliated band. It seems probable that the excretory cells were originally portions of the velar pre-oral ciliated band, and as their excretory functions for some reason became more and more important, they became separated from the velum and assumed a greater degree of development, eventually replacing the primitive "head-kidneys." The statements regarding the excretory cells of the Pulmonates are contradictory; but even if Bütschli and Fol should prove to be correct, and that in the forms they studied the vacuolated cells were really posterior to the pre-oral band, this may be regarded as a differentiation from a primitive condition which Rabl describes, and which exists in *Paludina*, *Bithynia*, and the Pteropods in which the vacuolated excretory cells form part of the pre-oral band. The fact, too, that in some Prosobranchs, *Fulgur* for instance, the excretory cells make their earliest appearance behind the velar ridge, is no objection to the idea advanced; it is readily explainable as an abbreviation of development, the original connection between the cells of the velar ridge and the excretory cells being dissolved at a very early stage.

Fol's idea that there is a serial homology between the excretory cells and the primitive or "head-kidneys" is entirely erroneous. So, too, Sarasin's conclusion that two pairs of excretory organs originally characterized the Molluscan veliger. No such arrangement has ever been observed in any of the larva or forms which show a relation by descent to the Trochophore.

V.—THE NERVOUS SYSTEM AND SENSE-ORGANS.

The first portions of the nervous system to make their appearance are the supra-œsophageal ganglia and the otocyst. The latter first becomes evident in *Fulgur* immediately below the excretory cells, but the exact mode of its formation I did not observe. The supra-œsophageal ganglia (Pl. XXIV, Figs. 10 and 11, *ce*) appear as a circular ectodermic thickening on either side in the anterior bend of the velar ridge, and therefore at the sides and a little dorsal to the mouth-invagination. In later stages (Pl. XXVII, Fig. 30, *ce*) the cells of this thickening proliferate, forming a mass projecting into the body-cavity on either side of the œsophagus, the cells of the thickening being to a certain extent loosely aggregated and having a very marked resemblance to the scattered mesoderm cells lying near. It is, in fact, impossible to distinguish the cells of the nerve-ganglion from the mesoderm cells by their shape or general appearance, and this has given rise to the numerous misconceptions which have arisen concerning the origin of the nervous system in the Mollusca. Early stages show conclusively, however, that the nerve-ganglion is an ectodermal structure, and is formed by a proliferation of the ectodermal cells, and not by an invagination.

The pedal ganglia arise in the same manner by a proliferation of the ectodermal cells of the foot (Fig. 30 *pg*), and soon assume a considerable size and come into contact with the otocysts, which then appear (*Ot*) to rest upon the ganglia. In the earlier stages no commissure could be detected between the supra-œsophageal and pedal ganglia, but in later stages cells from the former ganglia can be seen (Fig. 28) to grow downwards, so as to come into contact with the latter and so form the cerebro-pedal commissure (*cpc*). In like manner, in the early stages no commissures could be made out between the ganglia of opposite sides, but in later stages these commissures (*cc* and *pc*) were very distinct, as is shown by the sections (Figs. 29 and 31) through the supra-œsophageal (*ce*) and pedal ganglia (*pg*) of *Fasciolaria* in an embryo in which the gills had appeared.

Ziegler (31) seems to lay considerable stress on the development of the pedal ganglia in *Cyclas* in connection with the byssus-gland. In neither *Fulgur* nor *Fasciolaria* is this the case.

Fig. 30 shows the relationship of the byssus-gland (*by*) to the pedal ganglion (*pg*) of one side in *Fulgur* as seen on a sagittal section. The ganglion is seen to have originated at a point far distant from the orifice of the byssus-gland or *porus aquiferus*,[1] and it does not even lie in contact with the apex of that organ. The two figures of (Pl. XXV, Figs. 20 and 21) transverse sections through the foot of *Fasciolaria* show the same thing. Fig. 20 is through the foot, just below the apex of the byssus-gland (*by*). In *Fasciolaria* the gland divides about half-way from its orifice into two limbs the cut ends of which are shown in the section. On the sides of the gland-tubes can be seen a slight aggregation of cells (*pg*), which are the most posterior cells of the pedal ganglia. In the second section above this (Fig. 21) the closed apices of the gland-tubes can be seen (*by*), and outside these on either side are seen pedal ganglia (*pg*) much more distinct than in the preceding figure. But even here it is only the posterior portions of the ganglia which are seen, since the ganglia are present in no less than eleven sections anterior to the one shown in Fig. 21, the ninth section anteriorly being the one represented in Pl. XXVII, Fig. 29. It will be readily seen that in the Prosobranchs studied by me the pedal ganglia do not come into relation at all with the byssus-glands, lying in fact externally and anterior to their apices. That they do develop in close relation to the byssus-gland in some Lamellibranchs is certain from Ziegler's researches on *Cyclas*, already referred to, as well as from the more recent observations of Schmidt (25) on *Anodonta;* but it is also evident that there is no special significance in this relation, since it does not hold in the Gasteropods, nor, probably, even for all Lamellibranchs.

The eyes (*Oc*) arise as an invagination of the ectoderm of the head-vesicle at the bases of the tentacles (Pl. XXVI, Fig. 27). The invagination soon closes, and forms a shut sack the cavity of which remains visible for some time. The tentacles (*T*) are outgrowths of ectoderm, and are situated immediately above the

[1] I employ the term "byssus-gland" for the ectodermal tubular invagination found upon the foot of the Prosobranchs under discussion in preference to the term *porus aquiferus*, in consideration of the admirable researches of Barrois on "Les Glandes du Pied et les Pores aquifères chez les Lamellibranchs," Lille, 1885. There seems to be little doubt but that the *pori aquiferi* of the Gasteropods are homologous with the byssogenous glands of the Lamellibranchs.

supra-œsophageal ganglia, cells of which (*ce'*) pass into the hollow outgrowth and form the tentacular nerves.

I have no observations to offer on the development of the other portions of the nervous system, the oldest embryo which I preserved not having reached the stage in which these begin to differentiate.

My observations on the development of the nervous system of the Prosobranchs, meagre though they be, have suggested certain points for consideration. Hatschek has clearly demonstrated (36) the similarity of the Molluscan veliger to the Trochophore larva, and draws the conclusion therefrom that both Annelida and Mollusca, as well as certain other groups, have sprung from a common form presenting a structure very similar to that which characterizes the Trochophore larva, and which may be termed Trochozoon. The nervous system of the Trochozoon was an ectodermal thickening of the apical region of the body (the "Scheitelplatte"), which, becoming bilobed, is converted into the supra-œsophageal ganglia of the adult Annelid and Molluscan descendant. Does the development of the supra-œsophageal ganglia of the Prosobranch Molluscs bear out this supposition? Only with certain modifications. As above stated, the supra-œsophageal ganglia of *Fulgur* arise as two entirely separate ectodermal thickenings, lying not at the apical pole of the larva, but immediately in front of and at the sides of the mouth-invagination. At first sight they would appear to be structures quite distinct from the apical thickening of the Trochozoon, but it seems to me that an explanation can readily be found which will do away with the difficulties on this point, and at the same time will be borne out by what occurs in other groups of the Mollusca. There has been an abbreviation of the development, whereby the ganglia, instead of passing through the various phylogenetic stages which one might expect to find, form at once in the position which they occupy in the adult. What is meant can best be illustrated by comparing what occurs in an Annelid with what is found in *Fulgur*. Hatschek's description of *Criodrilus* (36) will serve the purpose. In the course of development the apical thickening increases, and sends down two processes towards either side of the mouth-opening, and so assumes a horse-shoe shape. The lateral processes, which are, like the

apical thickening, thickenings of the ectoderm, continue their growth downwards—*i. e.*, posteriorly—till they reach the sides of the mouth-opening, and finally unite behind the mouth, forming the sub-œsophageal ganglion. (See Fig. 15, Pl. II, of Hatschek's paper.) If now the portions of the lateral thickenings immediately before the mouth form directly, and not by a growth from a more anteriorly placed thickening, and at the same time the apical thickening disappears or does not form at all, the arrangement which obtains in *Fulgur* will result; that is, there will be two isolated ectodermal thickenings at the sides and a little in front of the mouth which only later become connected by a commissure.

If this be the true explanation, we might expect to find in some Mollusca traces persisting of the apical thickening. Are they to be found? I think so. In *Fulgur* it is doubtful whether there is any trace of the apical thickening; in an embryo I observed on surface view a dark patch, apparently a thickening, at the point where such traces might be expected, but sections of a stage of about the same age failed to give any definite evidence as to its significance. It is quite possible, however, that in the marine Prosobranchs the development may have been abbreviated to such an extent that no indication of the apical thickening persists—a conclusion which would be quite in accordance with other features, such as the disappearance of the "head-kidneys" which these forms exhibit. The Pulmonates, however, present in many points a more typical trochophore larva than do the Prosobranchs, and here we have a rudimentary structure which may be the remains of the apical thickening. In aquatic Pulmonates there is to be seen within the velar area a conspicuous mass of cells which are formed from the ectoderm in this region. Lereboullet first observed them (19), and they have since been described by Lankester (18), Fol (13), Rabl (22), and others. Lankester believed that they formed the supra-œsophageal ganglia; Fol, however, first showed that this was not the case, but that they do not give rise to any organ, but later become confused with the mesoderm. As to their significance he says: "Nous ne devrions, d'après cela, point les considérer comme le blastème d'un organe spécial et, pour expliquer leur constance et leur formation hâtive, nous serions contraints de supposer qu'il s'agit

ici d'un organe rudimentaire, c'est-à-dire d'un reste, transmis par hérédité, d'une partie qui, chez d'autres formes, posséderait une importance physiologique." Wolfson (30) advanced our knowledge of this problematic organ a little farther. He says concerning it: "Das wir es auch hier mit einem Nerven-Gebilde zu thun haben, unterliegt keinem Zweifel." And further: "Beim erwachsenen Thiere ist keine Spur mehr davon zu finden und glaube ich, dass es daher mit den übrigen Embryonal-Organen der Gasteropoden (Velum, Vornieren, Embryonal-Herzen, Schalengrabe, Nahrungssäcke) zusammenzustellen und als Embryonal-Hirn zu bezeichnen ist." He states, too, that in *Lymnæus* the ganglia arise as local thickenings of the ectoderm. We have accordingly in the aquatic Pulmonates an organ formed by a thickening and proliferation of the cells of the ectoderm in the apical regions which corresponds in its position and origin with the apical thickening of the Trochozoon, and which is entirely embryonic, disappearing in later life. It does not give rise to the supra-œsophageal ganglia, which arise as local thickenings of the ectoderm at the sides and above the mouth, but it has apparently something to do with the nervous system. I believe *this embryonic organ to be nothing else than the remains of the nervous apical thickening of the Trochozoon*, and consider the aquatic Pulmonates to show a stage in the abbreviation of the development intermediate between what occurs in the Archiannelida (*Polygordius*, *Criodrilus*, etc.), and in the Prosobranch Gasteropods.

In the other groups of the Mollusca on which we possess evidence at all certain on this point, we find they retain almost unaltered the original condition. Fol's researches on the Pteropods and Heteropods seem to indicate this, and the observations of Hatschek (15) and Ziegler (31) on the Lamellibranchs demonstrate it for that group. Schmidt's observations on *Anodonta* (25) seem to indicate that this form departs from the arrangement found in *Teredo* and *Cyclas*, and agrees more nearly with what is found in the Prosobranchs; this is probably, however, to be explained by the mode of life of the embryo *Anodonta*. It is interesting to note that the Pteropods, Heteropods and Lamellibranchs which show the greatest resemblance in other respects to what, it is to be believed, was the structure of the

original Trochozoon, also retain the apical thickening, and derive their supra-œsophageal ganglia from it, while the Prosobranchs which depart most widely from the Trochozoon do not show any sign of the thickening, and the Pulmonates, which, while possessing a rudimentary ciliary apparatus, yet retain the "head-kidney," and probably resemble the ancestral Trochozoon more nearly than the Prosobranchs, are intermediate, as regards the formation of the supra-œsophageal ganglion, between these and the Pteropods, etc.

The absence of a commissure between the ganglia of opposite sides at an early stage, and its formation later, receives its explanation in the idea that has been advanced above, and is, in fact, a necessary sequence of the processes which are supposed to have occurred.

As regards the pedal ganglia, their origin as independent proliferations from the ectoderm would seem to indicate that they are structures peculiar to the Mollusca, and are therefore not homologous with the subœsophageal ganglia of the Annelides. This idea has been advanced by one Ihering (29), who homologizes the visceral ganglia of the Mollusca with the ventral ganglionic chain of the Annelids. It does not seem that this homology will stand the test which comparative embryology affords. The researches of Hatschek, notably his recent observations on the development of the nervous system of *Polygordius* (38), demonstrate that in the more typical trochophore larvæ the subœsophageal ganglia develop in relation to the commissure thickenings of the ectoderm which extend downwards from the supra-œsophageal ganglia—*i. e.*, the apical thickening. If we are to accept the Trochozoon as the common ancestor of the Annelida and Mollusca—and the evidence we possess seems to make such a conclusion necessary—the pedal ganglia of the Mollusca must be homologized with the sub-œsophageal ganglia of the Annelides. In certain members of this latter group observations show that the ventral ganglia arise quite independently of the apical thickening and its commissural processes. Hatschek admits that such a process does occur. He says: "Bei directer Entwickelung mag es vielleicht in einzelnen Fällen vorkommen, dass die Schlundcommissur erst secundär Bauchmark und Scheitelplatte verbindet." If in the Annelids abbre-

viation of the developmental processes has produced the independent origin of the sub-œsophageal ganglion, it is exceedingly probable that the same phenomenon has occurred in the Mollusca, and accordingly there is no necessity for the supposition that either the pedal or supra-œsophageal ganglia of the Prosobranchs are entirely new structures unrepresented in the Annelids and their ancestors.

The derivation of the Mollusca from a Trochozoon ancestor renders improbable the suggestion advanced by Balfour (1) that the nervous ganglia of the Mollusca have been derived from a thickening of the walls of the sense-organs. The supposition that otocysts and visual organs have arisen in this group previously to the formation of a nervous system seems very improbable, and is not borne out by the facts of embryology. The nervous ganglia originate as thickenings of the ectoderm quite independent of the sense-organs, and only become connected with them later.

BALTIMORE, *April* 12, 1886.

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EXPLANATION OF PLATES.

Ad. Ad-oral cilia.
An. Anus.
by. Byssus-gland.
cc. Cerebral commissure.
cs. Supra-œsophageal (cerebral) ganglion.
cs'. Nerve-cells from supra-œsophageal ganglion which have passed into the cavity of the tentacle.

cpc. Cerebro-pedal commissure.
ect. Ectoderm.
el. Yolk-elevations.
end. Endoderm.
ex. Primitive excretory cells.
F. Foot.
HV. Head-vesicle.
inc. Cavity of invagination.
inv. Invagination of ectoderm.
M. Mouth.
me. Primitive mesoderm cell.
mes. Mesoderm.
N. Nucleus of excretory cell.
Oc. Eye.
Oe. Œsophagus.
Ot. Otocyst.
pb. Polar globule.
pc. Pedal commissure.
pg. Pedal ganglion.
Poo. Post-oral cilia.
pra. Protoplasmic aggregation.
Pro. Pre-oral cilia.
Sh. Shell.
T. Tentacle.
V. Velum.
Yk. Yolk-granules.

All figures, unless otherwise stated, are of *Fulgur*.

Plate XXIV.

Fig. 1.—Yolk-granules of *Fulgur* which have been treated with corrosive sublimate and alcohol, and subsequently with Schultz's solution.

Fig. 2.—Capsule of *Purpura floridana*.

Fig. 3.—Egg of *Fulgur* after extrusion of the polar globule.

Fig. 4.—Two-spheruled stage.

Fig. 5.—Four-spheruled stage.

Fig. 6.—Stage in which the protoplasm has aggregated at the formative pole for the formation of the micromeres.

Fig. 7.—Stage with eight micromeres.

Fig. 8.—Surface view of stage in which the primitive mesoderm cell is formed.

Fig. 9.—Surface view of formative pole of egg in which the ectoderm has been invaginated.

Fig. 10.—Surface view of stage in which the organs begin to form.

Fig. 11.—Surface view of stage a little later than the preceding.

PLATE XXV.

Fig. 12.—Section through an egg of stage represented in Fig. 6.

Fig. 13.—Section through a stage later than that represented in Fig. 7.

Figs. 14 and 15.—Sections through egg of stage represented in Fig. 8, showing the formation of the primitive mesoderm cell.

Figs. 16–19.—Sections through the ectodermal invagination seen in surface view in Fig. 9.

Figs. 20 and 21.—Transverse sections through the foot of an advanced embryo of *Fasciolaria.*

PLATE XXVI.

Fig. 22.—Diagram representing the arrangement of the cilia in a Prosobranch veliger.

Fig. 23.—Section through embryo in which the blastoderm has grown around the macromeres. Section is taken near one extremity of the embryo to show the continuation of the formation of protoplasmic aggregations.

Fig. 24.—Diagrammatic transverse section of the velum of a Prosobranch veliger. (This fig. and Fig. 22 are modified from drawings kindly furnished me by Dr. W. K. Brooks.)

Fig. 25.—Ventral view of an unidentified veliger.

Fig. 26.—Portion of excretory cells of *Fasciolaria.*

Fig. 27.—Horizontal section through tentacle and eye of *Fasciolaria* (advanced embryo).

PLATE XXVII.

Fig. 28.—Vertical section through the cerebral and pedal ganglia of an advanced embryo of *Fasciolaria.*

Fig. 29.—Transverse section through the pedal ganglia and pedal commissure of an advanced embryo of *Fasciolaria.*

Fig. 30.—Vertical section through ganglia of an advanced embryo of *Fulgur.*

Fig. 31.—Horizontal section through the region of the cerebral commissure of an advanced embryo of *Fasciolaria.* This section belongs to the same series and immediately succeeds that of which a portion is shown in Fig. 27.

THE ANATOMY AND DEVELOPMENT OF THE SALPA-CHAIN.

By W. K. BROOKS, Johns Hopkins University, Baltimore, Md. With Plates XXVIII and XXIX.

Notwithstanding the fact that most of the eminent students of marine animals have written upon the asexual multiplication of Salpa, our knowledge of the subject is still in hopeless confusion. The list of contributors to the voluminous literature includes the names of Chamisso, Cuvier, Eschricht, Quoy, Gaimard, Leuckart, Meyers, Huxley, Vogt, Krohn, Kowalevsky, Salensky, Todarro, and within the last three months a long illustrated paper has been published by Seeliger.[1]

During its early stages of development the proliferating stolon of Salpa is almost identical in structure with that of Pyrosoma, and there is therefore every reason for believing that the process of budding is essentially the same in both of them, and that a thorough knowledge of the history of the two forms will at least furnish a basis for comparing the process of asexual multiplication in the one with that in the other; but any attempt to make a detailed comparison between any one of the conflicting accounts of the origin of the Chain-Salpæ, and our knowledge of Pyrosoma, is totally useless.

This difficulty is not due to imperfect knowledge of Pyrosoma, for we have two minute and amply illustrated papers on the budding of Pyrosoma, one by Huxley and one by Kowalevsky; and as the Russian embryologist confirms, in every particular, the simple and consistent history which Huxley had traced many years before, we may safely accept their account, and decide that the process of budding in Pyrosoma is accurately known, so far, at least, as its general features are concerned. It is quite possible that new contributions to its minute histology may be made in the future, but the general history is well known.

Reasoning from what is known to what is still unknown, we

[1] Die Knospung der Salpen, von Oswald Seeliger. Jen. Zeitsschrift f. Naturwissenschaft, Bd. XIX, N. F. XII, 1885.

may feel a reasonable hope that a thorough acquaintance with the history of the Salpa-stolon will enable us to show that the process of budding is essentially like that which occurs in Pyrosoma, but the attempt to reconcile the various accounts with each other or with our knowledge of Pyrosoma leads only to the most hopeless confusion; and there is every reason for believing that this is due to our ignorance regarding the process in Salpa.

The modern method of studying embryological anatomy by sections must lead to most perfect and decisive results when the sections are correctly interpreted; but unfortunately a fundamental error in the interpretation of the sections leads us away from the truth with equal certainty, and the railway traveler who enters the wrong train is not hurried more swiftly and surely astray than the embryologist who makes an error of this sort, for which, in the case of complicated organisms, there are many opportunities.

This paper is an abstract of an illustrated one in which I shall show that the budding of Salpa is, in reality, a very simple process, directly comparable with the budding of Pyrosoma, and that all the recent writers upon the subject—the latest contributor, Seeliger, as well as the others—have gone completely astray in the interpretation of their sections.

In 1880 I asked Prof. Spencer F. Baird to supply me with specimens of Salpa, that I might study the origin of the eggs. He accordingly sent me a number of specimens of two species which were collected in the summer of 1880 at the station of the United States Fish Commission at Wood's Hole. One of them is the small Salpa Cabotii, which is frequently found in abundance on our coast. It is very similar to Salpa democratica-mucronata.

The other species, which is much larger, may possibly be new, although I find, in my preserved specimens, no reason for questioning its identity with Salpa pinnata, a species which has never been recorded as occurring on our coast. As soon as I received the specimens, Dr. I. Bermann kindly aided me by cutting several thousand sections, and my observations on the origin of the eggs, based upon these sections, were published soon after.

My examination of the sections, and of others which were afterwards made for me by Dr. Chas. S. Dolley, convinced me that the true nature of the Salpa-chain has never been compre-

hended; that the differences between the process of budding in Salpa and our knowledge of the process in other Tunicata have been greatly overestimated, and that the stolon of Salpa is essentially like that of Pyrosoma as regards its structure, its origin, and its whole history, and that the differences are superficial and of minor importance.

The young Pyrosoma is not produced by budding from the wall of the stolon, but by the gradual development of the tissues of each segment of the stolon into the organs and tissues of the new organism. The stolon becomes converted into a single series of animals, which are placed dorsum to venter, with their neural surfaces towards the base and their hæmal surfaces towards the tip of the stolon. Their right sides all arise on the right half of the stolon, and the plane which divides each segment of the young stolon into symmetrical halves is the same as the median plane of the body of the young ascidian.

The young Salpa-stolon is bilaterally symmetrical, like that of Pyrosoma, but the mature Salpa-chain consists of two rows of ascidians placed with their hæmal surfaces towards the middle line of the stolon, and their neural surfaces external; the right sides of all which lie on the left side of the stolon, and the left sides of all the others, are towards its base.

My sections showed that this condition of things is the result of crowding and pressure, and that there is really only a single series, as Seeliger also has recently shown. My sections showed, however, that not only the presence of two rows of Salpæ, but also the relative positions which the animals occupy in advanced chains, are the results of rotation produced by crowding, and that after we have interpreted all the secondary changes which are thus produced, the Salpa-chain is found to be not only a single series of animals, but a single series placed dorsum to venter, with the neural surface of each one turned towards the base of the stolon, and the right sides of all on the right side of the stolon; and that, exactly as in Pyrosoma, the middle plane of symmetry in the young stolon coincides with the middle plane of the body of each Salpa.

My interest in other subjects, and my inability to secure the publication of the figures which I had drawn, prevented me from publishing my results, and the subject remained incomplete

until my attention was recalled to it two months ago by the appearance of Seeliger's paper on the budding of Salpa.

My first hasty examination of this paper led me to believe that Seeliger had solved the problem, since he shows that the stolon gives rise to only a single series of Salpæ, and that the animals are pushed, as they increase in size, alternately to the right and to the left, until what is apparently a double series is produced by crowding, as I had also discovered.

More careful examination of his paper showed me, however, that with this exception his results are totally irreconcilable with my own, or with our knowledge of the budding of Pyrosoma, since Seeliger believes that the median plane of the stolon is, from the first, at right angles to those of the Salpæ; and that the right half of the stolon, instead of giving rise to the right halves of the bodies of all the Salpæ, produces the neural portions of the bodies of the 1st, 3d, 5th, and so on, and the hæmal portions of the bodies of the 2d, 4th, 6th, etc.

In Pyrosoma the bodies of the series of ascidians arise in the following relation to the middle plane of the stolon (see Fig. 1), *P* indicating the proximal end and *D* the distal end of the stolon; and *n* the neural surface, *h* the hæmal surface, *r* the right side, and *l* the left side of each zooid, and the dark line the median plane of the stolon. My studies had convinced me that this is true of Salpa also, but according to Seeliger the primitive or true relation of parts in the Salpa-stolon is like Fig. 2.

I therefore returned to the study of my specimens with renewed interest, and I have spent the last two months in a review of the subject, and I have obtained from the larger species a series of sections which show in the most satisfactory and conclusive manner that the Salpa-stolon is directly comparable, in every respect, with that of Pyrosoma, and that the secondary complications which gradually make their appearance as development progresses, on account of the pressure of the animals upon each other, are of such a character that it is very difficult to trace or even to discover them from the study of transverse sections of the stolon, while they are clearly shown and easily understood in horizontal sections—that is, in sections parallel to the long axis of the stolon, and therefore transverse to the long axes of the bodies of the Salpæ.

As the whole art of preparing serial sections has been remodeled since 1880, I now find no difficulty in cutting and mounting, in their proper places, long horizontal sections of advanced stolons; for, although the bodies of the constituent Salpæ

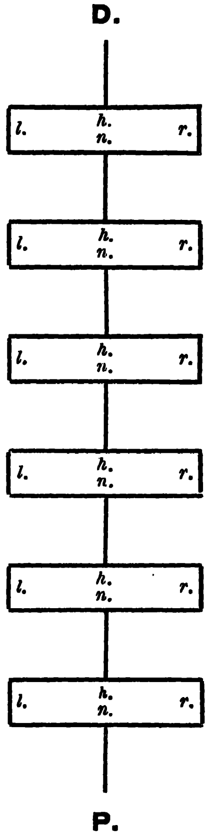


FIG. 1.

Diagram to show the normal or primitive positions of the Salpæ with reference to the long axis of the stolon. The black line is the middle line of the stolon; *P*, its proximal end; *D*, its distal end; the rectangles represent the bodies of the Salpæ; *r*, their right sides; *l*, their left sides; *h*, their hæmal surfaces, and *n*, their neural or cloacal surfaces.

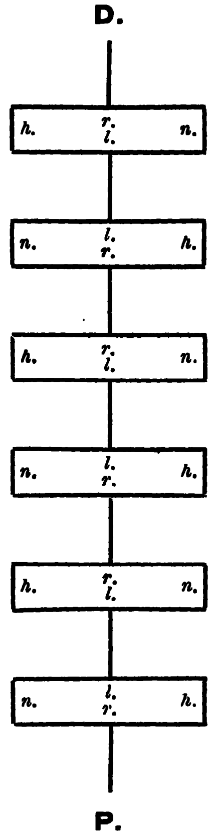


FIG. 2.

Diagram to represent Seeliger's view of the manner in which the Salpæ are formed upon the stolon. Reference letters as in Fig. 1.

are free from each other, they remain in their proper places on the slide, and a consecutive series of these horizontal sections of the stolon reveals its whole structure and the mutual relations of all its constituent elements with a clearness which could never be attained by the study of transverse sections alone, for the horizontal sections show that the body of each Salpa gradually undergoes a spiral twist around the long axis of its body, so that the planes of sections parallel to this axis—that is, transverse sections of the stolon—are changing continually. This change is of such a character that the relative positions of the various organs of the body are quite unlike in the successive sections of a series from the body of a single Salpa transverse to the stolon, and corresponding sections of successive stages of growth are still more confusing. There is no such difficulty in the study of horizontal sections of the stolon, for as these cut the body of each Salpa perpendicular to its long axis, the twisting of the body around this axis is easily intelligible.

The various writers who have failed to discover this secondary twisting of the bodies, and have accordingly interpreted their sections of the younger stages as if the relative positions of parts in the young buds were the same as they are in advanced embryos, have inevitably been led to conclusions which are totally erroneous. Seeliger, who relies on transverse sections, has failed completely, like all the others, and his account of the process of bud-development is of no more value than those given by earlier writers. In fact, all the writers who have recently studied the Salpa-stolon are totally and hopelessly astray, and none of the published accounts of the minute details of the process of bud-development have any value whatever. I shall therefore make no attempt to reconcile their conflicting accounts with each other or with the facts, but shall describe the phenomena as I have found them.

When viewed from above or from below, the bodies of the series of Salpæ in an advanced chain are arranged in the way which is shown in Fig. 3. They form two rows, one (*a*) on the right side of the long axis of the stolon, and the other (*b*) on the left; their hæmal surfaces (*h, h, h, h, h, h*) are on the middle line, and their neural surfaces (*n, n, n, n, n, n*) are external, while the left sides (*l, l, l*) of the bodies of all which lie on the right side of the stolon, and the right sides (*r, r, r*) of those on its left, are

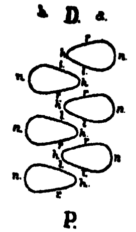


FIG. 3.

Diagram of the oral ends of the bodies of six of the Salpæ in a mature chain, to show their relative positions. *P*, proximal end of stolon; *D*, distal end of stolon; *a*, the series of Salpæ on the right side of the chain; *b*, the series of Salpæ on the left side of the chain; *n*, *n*, *n*, neural surface of the body of the chain-Salpa; *h*, *h*, *h*, hæmal surface; *r*, *r*, *r*, right side of Salpa; *l*, *l*, *l*, left side of Salpa.

directed towards its base or proximal end (*P*). The left side of each Salpa is adjacent to the right side of the adjacent Salpa on the same side of the stolon, and also adjacent to the left side of the adjacent Salpa on the opposite side of the stolon.

Todarro and Salensky believe that this, the final position of the individuals, is the position in which they arise on the stolon, while Seeliger correctly shows that the double series is secondary. He believes, however, that they arise on the stolon in the positions which would be occupied by the embryos in Fig. 3 if they were driven together into a single series. Figure 4, which I have abstracted from Fig. *B*, on page 30 of his paper, will serve to illustrate his view. He holds that the neural portions (*n*, *n*, *n*, Fig. 4) of the bodies of one-half the Salpæ, and the hæmal portions (*h*, *h*, *h*) of the bodies of the other half, arise on the same side of the stolon; and that the right sides (*r*, *r*, *r*) of all which have their neural surfaces on the left side of the stolon, and the left sides of all the others, are proximal in their origin.

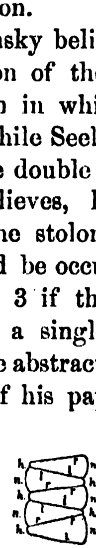


FIG. 4.

Diagram to illustrate Seeliger's view of the origin of the Salpæ on the stolon. *n*, *n*, *n*, neural surfaces; *h*, *h*, *h*, hæmal surfaces; *r*, *r*, *r*, right sides; *l*, *l*, *l*, left sides.

He therefore derives the organs of the hæmal portions of the bodies of one-half of the Salpæ, and the organs of the neural portions of the bodies of the other half, from one side of the stolon.

I shall show that this account of the process is totally erroneous, and that we have in Salpa, as in Pyrosoma, a single series (Fig. 5); that all the neural surfaces (*n*, *n*, *n*, *n*, *n*) are prox-

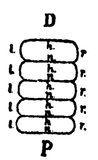


FIG. 5.

Diagram of a portion of a young stolon, to show the primitive positions of the Salpæ; letters as in Fig. 4.

imal in their origin, and all the hæmal surfaces (*h*, *h*, *h*, *h*, *h*) distal; while the right halves of the bodies of all (*r*, *r*, *r*, *r*, *r*) arise on the right half of the stolon, and their left halves on the left; that corresponding parts of all the ascidians in the series arise in Salpa, as they do in Pyrosoma, in corresponding positions upon the stolon; that the middle plane of symmetry in the Salpa-stolon is identical, like the middle plane of the stolon of Pyrosoma, with the middle planes of the bodies of all the ascidians in the series; and that the final position of the bodies of the chain-Salpæ, which is shown in Fig. 3, is the effect of two secondary changes in position, one of which has been detected by Seeliger, while he has failed to discover the other, just as Todarro, Salensky and other writers have overlooked both of them.

During the development and growth of the chain the constituent Salpæ are crowded alternately to the right and to the left, as Seeliger has shown, and while this change is taking place each Salpa rotates upon its long axis until its hæmal surface becomes internal and its neural surface external, and the position shown in Fig. 3 is assumed.

This rotation does not affect all parts of the body at the same time. The oral ends of the Salpæ, with their ganglia, are the first portions of the bodies to push out towards the sides and to form an alternating double row, but they are the last to rotate. The nuclear or ab-oral ends of the bodies are the first portions to become free from each other and from the wall of the stolon, and the rotation begins at the nuclear end of each Salpa at a very early stage of its development, and gradually extends upwards towards the oral end as development progresses and the body becomes more and more completely separated from the stolon. The oral ends are last affected, and for a long time after the nuclei, the cloacæ, the gills, and the greater part of each branchial sac are in their final positions, the oral ends of the bodies, although they form an alternating double row, still have all their right sides towards the right side of the stolon, and all their ganglia towards its proximal end.

Fig. 12, which is a slanting horizontal section of a stolon at this stage, cutting the bodies of the chain-Salpæ transversely at higher and higher levels, exhibits the extent and character of the

process of rotation. As the thickness of the body of a chain-Salpa from side to side is considerably less than its depth on the middle line, a series placed side by side can be packed along a much shorter stolon than that which would be required if they were placed dorsum to venter, in the positions which they occupy when young, and the rotation of their bodies therefore serves, like the formation of a double row, to increase the number which can be developed at one time upon the stolon.

After all the secondary changes which are thus brought about are eliminated, the Salpa-stolon becomes directly comparable with that of Pyrosoma, and as there are no secondary changes in Pyrosoma, the clearest and most intelligible method of presenting the facts is to begin with a short account of the Pyrosoma-stolon, as the simplest expression of the phenomena in question, and then to describe the various changes through which this becomes converted into the complicated Salpa-chain.

The egg of Pyrosoma gives rise to a rudimentary ascidian or cyathozooid; this develops a stolon which becomes converted into a chain of four ascidizooids, each of which produces a stolon from which other ascidizooids are formed, and each one of these gives rise to a stolon from which other ascidizooids are formed, and so on indefinitely.

Fig. 6 is a diagram copied from Kowalevsky's Fig. 7, and it represents a young stolon as seen in longitudinal section, while Fig. 7 is a copy of his figure of a transverse section of the same stolon. It consists—1st, of an ectodermal tube (*ec*) derived from the ectoderm of the hæmal surface of the body of the parent (*P*); 2d, of an endodermal tube (*en*) the cavity of which is a continuation of the branchial cavity or pharynx (*b*) of the parent, while its wall is derived from the endoderm of the fold between the halves of the parental endostyle (*es*); 3d, of two cloacal tubes (*c*), one on each side; 4th, of a nerve-tube (*n*), and 5th, of an ovary (*o*), which is derived from the ovary (*o*) of the parent (*P*). The stolon produced by the cyathozooid, those produced by the four primary ascidizooids, and those produced by the numerous secondary ascidizooids, are essentially alike; in each case the stolon arises on the hæmal surface of the body; in each case its endodermal tube opens into the branchial sac, and its wall comes from the fold between the halves of the endostyle, and in each case its

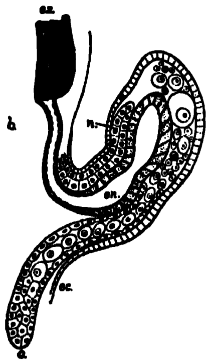


FIG. 6.

Longitudinal section of the young stolon of Pyrosoma, from Kowalevsky. *ec*, ectoderm. *en*, endoderm; *n*, nerve tube; *o*, ovary; *P*, parent; *b*, branchial sac of parent; *es*, endostyle of parent.

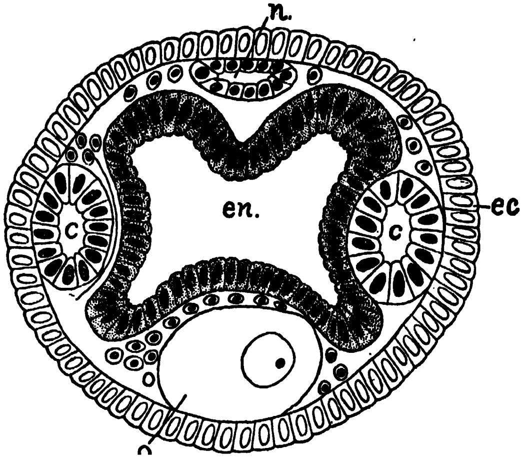


FIG. 7.

Transverse section of the young stolon of Pyrosoma, from Kowalevsky. *ec*, ectoderm. *en*, endoderm; *n*, nerve-tube; *c*, *c*, cloacal tubes; *o*, ovary.

transverse section is essentially like Fig. 7, except that no ovary has as yet been discovered in the stolon which is produced by the egg-embryo or cyathozooid.

In each case the stolon lengthens and soon becomes divided by circular constrictions into a series of segments, each of which gradually assumes the characteristics of an ascidizooid, as shown in Fig. 8.

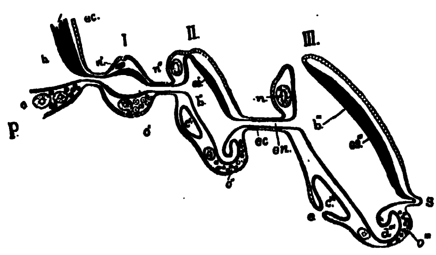


FIG. 8.

Diagrammatic longitudinal section of an advanced Pyrosoma-stolon, compiled from the figures and descriptions given by Huxley and Kowalevsky. *P*, parent; *I*, *II*, *III*, three segments of the stolon at successive stages of development; *b*, *b'''*, *b'''*, branchial sacs; *c''*, *c'''*, cloacæ or median atria; *e*, excurrent aperture of cloaca; *ec*, ectoderm; *en*, endoderm; *es*, *es''*, *es'''*, endostyles; *n''*, *n'''*, ganglia; *o*, *o'*, *o''*, *o'''*, ovaries; *d'''*, stomach; *s*, stolon.

This figure is a diagram compiled from Huxley's figures and description, and from Kowalevsky's Taf. XXXVII, Fig. 7, and Taf. XLI, Fig. 56. The gill-slits and the lateral atria are omitted, and the outlines of the bodies are a little altered, in order to facilitate comparison with Salpa, but the relations between the various parts will be found, upon examination, to be as they are described and figured by the two writers on Pyrosoma.

The stolon soon becomes enlarged at its tip, and the terminal segment (or in the case of the stolon produced by the cyathozooid each one of the four segments) gradually becomes converted into an ascidizooid (Fig. 8, III) which is placed with its right side on the right side of the stolon, its neural surface (n) towards the base of the stolon, or towards the hæmal surface of the parent (P).

The plane of symmetry in the stolon is the same as the middle plane of the body of the young bud and that of the parent, and the various regions of the body of the bud are placed like those of the parent. The branchial sac (b''') of the bud communicates, through the endodermal tube of the stolon, with the branchial sac (b) of the parent (P). This tube, which arises between the halves of the endostyle (es) of the parent, opens into the neural surface of the branchial sac of the bud, while the endostyle (es''') is developed upon its opposite or hæmal side. The digestive tract is formed as an outgrowth or diverticulum (d''') from the right half of the ab-oral end of the branchial sac, and the cloaca (c''') is formed by the union of the two cloacal tubes of the young stolon. It ultimately opens to the exterior on the neural surface of the body at c.

After the terminal bud (III) is well advanced, the second bud (II) also begins to assume the organization of an ascidizooid, and each stolon usually presents three or more segments in various stages of development, the terminal one being most advanced. In the stolon produced by the cyathozooid, however, all four segments develop simultaneously. In the latter case, however, as well as in the ordinary stolon, all the animals are placed in the same position, and this is directly comparable with the position of the animal which produces the stolon. The right and left sides of all the buds are respectively on the right and left sides of the stolon; their nuclear or ab-oral ends corre-

spond with each other, and with the nuclear end of the parent; their oral ends are all similarly placed; their neural surfaces are all proximal, and all the hæmal surfaces are distal or towards the tip of the stolon, as is also the hæmal surface of the parent. Each of the animals in the series, the parent as well as all the others, is joined to the body of the next one by a double tube or stolon, which consists of an outer wall of ectoderm and an endodermal tube the proximal end of which arises between the folds of the endostyle on the hæmal surface, while its distal end joins the neural surface of the next animal in the series on the middle plane of its neural surface. By comparing Fig. 8 with Figs. 6 and 7, it will be seen that the various structures which are revealed in a transverse section may be divided into two classes, with reference to their history: the nerve-tube (*n*), the two cloacal tubes (*c*, *c*) and the ovary (*o*) very quickly become divided into portions which become entirely independent of each other, and give rise to the ganglia (Fig. 8 n^1, n^2, n^3), the cloacæ or median atria (c', c''), and the ovaries (o^1, o^2, o^3) of the young ascidians, while the ectodermal tube (*ec*) and the endodermal tube (*en*) persist for a much longer time, and do not become discontinuous until the young ascidian is set free at the end of its embryonic life. Up to this period its branchial sac maintains its channel of communication with that of its parent, and the blood-cavities are also in communication with each other.

The process of bud-development in Pyrosoma, therefore, is not a process of budding from the walls of the stolon, but it consists in the direct conversion of the segments of the stolon into the bodies of the new organisms: and the advanced stolon, at the stage shown in Fig. 8, consists of a series of slightly modified tubular portions, alternating with portions which are undergoing development into ascidians; it is therefore divided up, from base to tip, into a series of alternating segments, which are alternately unmodified and developed into new animals. In the ordinary stolon the ascidians are close together, and the unmodified portions of the tube are short; and I have in the diagram lengthened them in order to facilitate comparison with Salpa. In the stolon which is produced by the cyathozooid they are, however, much longer than I have represented them in the diagram, as will be seen by a reference to Kowalevsky's Fig. 56. Careful study of

this figure, and of the various figures and the descriptions given by Huxley and Kowalevsky, will also show that, in this case as in the ordinary stolon, the connecting tubes are hæmal in their origin and neural at their distal ends, and that the relation of each one of the four primary ascidizooids to the others, and to the parent cyathozooid, is precisely as it is in the ordinary stolon.

Before an ascidizooid like the one shown at III in Fig. 8 is set free, the stolon by which it is destined to produce new organisms like itself can be found at *s* upon its hæmal surface, composed of an ectodermal sheath, and of an endodermal tube which arises between the halves of its endostyle; and after No. III in the figure is set free, the tube which had connected it to No. II furnishes the basis for the stolon of No. II.

It will therefore be seen that the process by which the four primary ascidizooids are developed from the egg-embryo, that by which secondary ascidizooids are developed from the primary ascidizooids, and that by which the successive generations arise from the bodies of the secondary ascidizooids and their descendants, is the same throughout; and that each one arises in the same manner and in the same relation to the one before it in the series as all the others.

In fact, the whole Pyrosoma-community or ascidiarium may be regarded as a single stolon with discontinuous development.

Turning now to Salpa, we have a life-cycle which consists, first, of the solitary Salpa hatched from the egg, and, secondly, of chains of Salpæ which are developed from the proliferating stolon. The stolon (which is shown at a very young stage in longitudinal section in Fig. 9, which is copied from Seeliger's Fig. 2, Taf. 1, and in transverse section in Fig. 10, which is drawn from one of my own sections) arises from the hæmal surface of the solitary Salpa, and consists of an outer wall of ectoderm (*ec*); an inner endodermal tube (*en*), which opens into the branchial sac of the solitary Salpa and arises in the fold between the halves of its endostyle; a nerve-tube (*n*), which quickly divides up into a series of ganglia (Fig. 11 *n*, *n*, *n*) which develop discontinuously; an ovary (*o*), which gives rise to the single ovum (*o′*, *o′*, *o′*) which is found in each chain-Salpa, and two cloacal tubes (*c*, *c*), which divide up, at a very early stage of

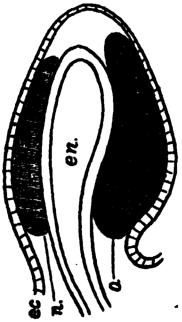


FIG. 9.

Longitudinal section of a young Salpa-stolon, from Seeliger's Plate I, Fig. 2. *ec*, ectoderm; *en*, endoderm; *n*, nerve-tube; *o*, ovary.

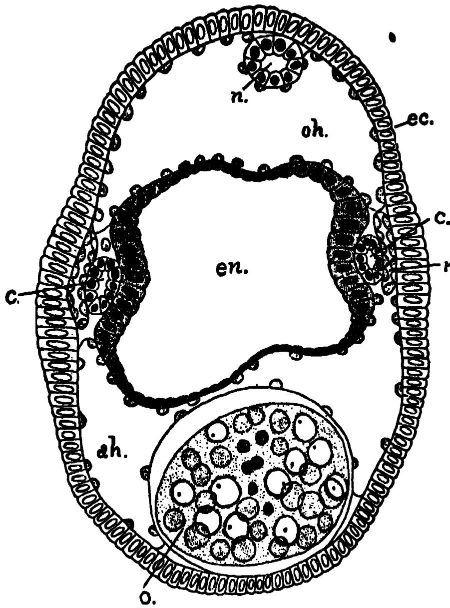


FIG. 10.

Transverse section of a young stolon of Salpa. *c*, *c*, cloacal tubes; *ec*, ectoderm; *en*, endoderm; *m*, mesodermal tubes; *n*, nerve-tube; *oh*, oral hæmal tube; *ah*, aboral hæmal tube; *o*, ovary.

development, into two series of closed vesicles, which ultimately unite on the middle line of each chain-Salpa to form its cloaca (c', c', c') or median atrium. There are two minor differences between the young stolon of Salpa and that of Pyrosoma: the whole ovary (Fig. 9 *o*) is contained within the stolon, and there are two tubular masses of cells (*m*, *m*) which form the muscles of the Salpæ, and have never been detected in the Pyrosoma-stolon.

The Salpa-stolon soon becomes greatly elongated, and divides up into a very great number of chain-Salpæ, which form a single series placed in corresponding positions, as shown in Fig. 11, each joined to the one beyond it by a tube of ectoderm which contains an endodermal tube arising on the hæmal surface between the halves of the endostyle, and joining the neural surface of the next one in the series. The middle plane of the symmetrical stolon is the same as the middle plane of the body of the solitary Salpa, and those of all the chain-Salpæ; the right

side of the solitary Salpa and the right sides of all the zooids are on the right of the stolon; the hæmal surfaces of all are towards the tip of the stolon, their neural surfaces turned away from the tip, and their oral and ab-oral ends are all in corresponding positions.

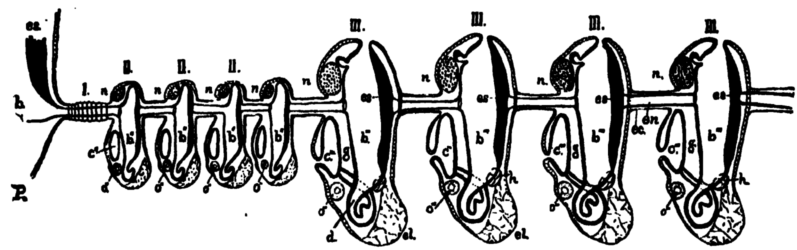


FIG. 11.

Diagrammatic longitudinal section of an advanced Salpa-stolon, showing the constituent Salpæ in the positions which they would occupy were there no secondary changes of position during growth. *P*, parent or solitary Salpa; *I*, the youngest and most proximal chain; *II, II, II, II*, the series of ascidians which makes up the second chain; *III, III, III, III*, the series of ascidians which forms the oldest chain; *b″*, *b‴*, branchial sacs; *c″*, *c‴*, the cloacæ or median atria; *d″*, *d‴*, the digestive tracts; *ec*, ectoderm; *el*, eleoblasts; *h*, heart; *en*, endoderm; *es*, endostyle; *g*, gill; *n*, ganglia; *o″*, *o‴*, ova.

The relation which the solitary Salpa bears to the first chain-Salpa in the series (the last which it produces) is exactly the same as the relation between this one and the next, and the solitary Salpa is as strictly a member of the series as any of the others, and although it is much more mature than any of the others, the development of its stolon begins while it is still an embryo.

As is the case with Pyrosoma, the segment at the tip of the stolon is much more advanced than the one at the base, and it is set free while the latter is still embryonic; but while the stolon of Pyrosoma (Fig. 8) presents a series of three or four zooids in successive stages of development, the stolon of Salpa presents us with a series of sets of zooids, from fifty to a hundred in each set, all those in one set being at nearly the same stage of development, and the most distal set the oldest.

The diagram represents only four in each set, as these are enough to exhibit their relation to each other; and I may also state here that the zooids which make up each set are not always in exactly the same stage of development. In fact, there is,

during the young stages, a marked gradation, the distal members of the set being more advanced than the proximal ones; nor is the transition from one set to the next abrupt, as shown in the diagram, the distal member of one set being separated from the proximal member of the next set by a few intermediate or transitional zooids.

Owing to the very great number of zooids in the series, they are packed so closely that the connecting tubes are very short, but I have lengthened them in the drawing for convenience in lettering, and also in order to express more clearly the fact that the Salpæ are not formed as buds from the wall of the stolon, but by the direct development and metamorphosis of its constituent tissues and cavities, exactly as is the case in Pyrosoma.

The diagram represents the stolon as it would be if there were no secondary changes, due to crowding and pressure; but long before the bodies of the chain-Salpæ are as well developed as they are represented in the second set in the diagram, the secondary complications begin to manifest themselves. The nerve-tube divides at a very early stage into a series of closed vesicles each of which ultimately becomes the ganglion of a Salpa. Fig. 10 shows that the nerve-tube is at first on the middle line of the oral surface of the stolon, above the digestive tube, but as soon as it divides into segments these push alternately to the right and to the left, finally descending to the level of the digestive tube or a little below it, and forming two rows, one on each side of the stolon, so that if this change were represented in side view in the diagram (Fig. 11), the connecting tubes between the Salpæ should be represented as alternately hidden behind and hiding the ganglia, as shown in horizontal section in Fig. 14, while Fig. 15 shows the appearance which would be presented by a horizontal section or an oral view of Fig. 11. The oral ends of the bodies undergo no other change until the chain is nearly mature and almost ready to be discharged, but the ab-oral ends of the bodies not only crowd alternately to the right and to the left, like the oral ends, but also become twisted upon themselves around their long axes in such a way as to carry their neural or cloacal surfaces outwards.

This twisting begins at the ab-oral or nuclear ends of their

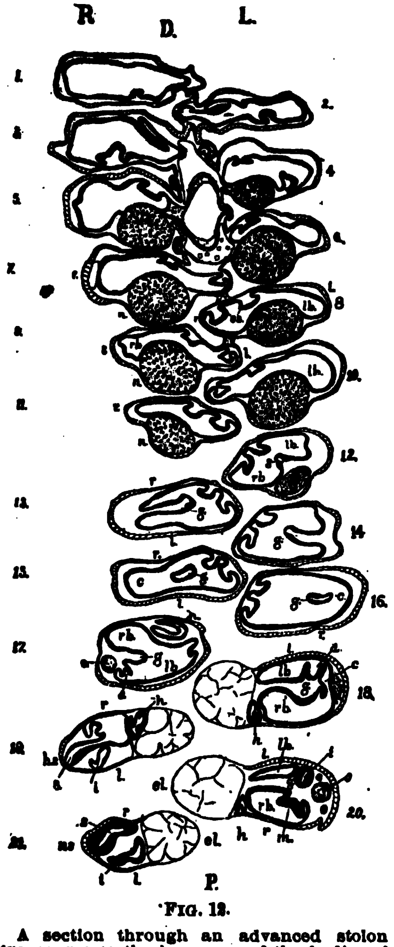


FIG. 12.

A section through an advanced stolon transverse to the long axes of the bodies of the Salpæ, and inclined to the long axis of the stolon, so that the Salpæ 20 and 21, at the proximal end of the series, are cut through their nuclei and their eleoblasts, while those at the other end of the series (1 and 2) are cut through their oral ends. The section was mounted with the oral surface below, and the right half of the stolon *R* is therefore on the left side of the figure. *P*, proximal end; *D*, distal end; *L*, left, and *R*, right side of stolon; *l*, left side, and *r*, right side of each Salpa. If there were no displacement, the Salpæ would form a single series of 21 animals, as numbered, and the odd numbers have become crowded into the right side, and the even ones into the left by the pressure of adjacent Salpæ. *a*, anus; *c*, cloaca; *el*, eleoblast; *es*, endostyle; *g*, gill; *h*, heart; *i*, intestine; *lb*, left half of branchial sac; *rb*, right half of branchial sac; *l*, left side of Salpa; *r*, right side of Salpa; *o*, ovum; *s*, stomach; *m*, mouth; *n*, ganglia; *ns*, neural surface.

bodies as soon as these are developed, and it keeps pace with the gradual growth of the Salpæ, so that that portion of the body of each Salpa which lies below the level of the stolon has one of its sides towards the proximal end of the stolon, the other side distal, and its hæmal surface towards the middle line of the chain; while the portion of the body which lies upon the level of the stolon or above it retains for a long time a position which is nearly normal, with its right side towards the right side of the stolon, and its neural surface towards the base of the stolon.

Fig. 12 is a horizontal section through a stolon in which the bodies of the Salpæ have pushed to the sides, thus forming a double row, and in which the lower or nuclear ends of the bodies have rotated, so that their cloacal or neural surfaces are external, while their upper or oral ends are more nearly in their primitive positions, with their ganglia (*n*, *n*, *n*) towards the base of the stolon, and the right sides of all (*r*, *r*, *r*, *r*, *r*) towards the right side of the stolon. As the stolon is not straight, but curled into the arc of a circle, a section which cuts the bodies of some of the

Salpæ transversely at the level of the stolon, will cut others at lower and lower levels, so that a single section like the one which is figured not only shows the relation of the bodies to each other, but it also gives such a series of transverse sections of the constituent Salpæ as is equivalent to a consecutive series of transverse sections of a single one.

The section which is figured slants upwards distally, so that the animals nearest the proximal end (*P*) of the stolon (21 and 20) are cut through their nuclei and eleoblasts; those which are a little more distal (15 and 16) through their branchial sacs, gills and cloacæ; those a little higher up (10 and 11) through their ganglia (*n*, *n*); 7 is cut through its line of union with the ectoderm of the lower surface of the stolon; 6 through its line of communication with the lower hæmal tube of the stolon; 5 through its area of communication with the endodermal tube of the stolon; 4 and 3 through their communication with the upper hæmal tube of the stolon; 2 through the area of union with the ectoderm of the stolon; and 1 is cut above or on the oral side of the stolon.

As the series to which the section belongs was begun at the ab-oral surface, the Salpæ are placed with their nuclear or ab-oral ends towards the observer, and their oral ends below the plane of the paper. The right side (*R*) of the stolon is therefore on the left of the figure, the small letters *l* and *r* indicating the left and right sides of each Salpa. Owing to the curvature of the stolon, no two of the Salpæ are cut in perfectly parallel planes, and the successive sections become more and more oblique as we pass from *D* to *P*. The greater distance between the bodies of the Salpæ at the proximal end (*P*) of the series is another effect of the curvature of the stolon. Between No. 1 and No. 7 the Salpæ lie so close together that there is no room for the reference-letters, and I therefore give another figure (13), representing Nos. 3–7 of Fig. 12 in their natural relation to each other and to the stolon, except that the connecting tubes of the stolon are represented as lengthened.

The section numbered 21 passes through the eleoblast (*el*), the stomach (*s*) and the intestine (*i*); the middle plane of the body is at right angles to the long axis of the stolon, the neural surface (*ns*) external, and the left side proximal. In the next

four sections (20, 19, 18, 17) the œsophagus is shown opening at the mouth (*m*) into the right half of the branchial sac (*rb*), which is separated from the left half (*lb*) by the base of the gill (*g*). In these four sections the heart (*h*) is also shown on the right side of the hæmal end of the body. The intestine (*i* 20, 21) opens through the anus (*a* 17 and 18) into the cloaca (*c* 18), on the left side of the gill (*g*). The egg (*o*) is shown in 17 and 20. Figs. 15 and 16 still have their neural surfaces outwards and the middle plane at right angles to the long axis of the stolon, but the endostyle is oblique, its right half in Fig. 16 and left half in Fig. 15 inclining towards the far side of the stolon. This is the first indication which is met with at the ab-oral end of the body of the primitive position of the Salpæ on the stolon, and careful examination will show that it is the left halves of the endostyles of the odd numbers which are on the right side (*R*) of the stolon, and the right halves of the endostyles of the even numbers, which begin to push in towards the opposite side; and, if the series from 14 to 6 be carefully studied, it will be seen that the left halves of all the endostyles ultimately pass into the left side, and the right halves into the right side. The series also shows that all the hearts are adjacent to the right halves of the endostyles, and the study of younger stages shows that all the hearts and all the stomachs and intestines are derived from the right half of the stolon.

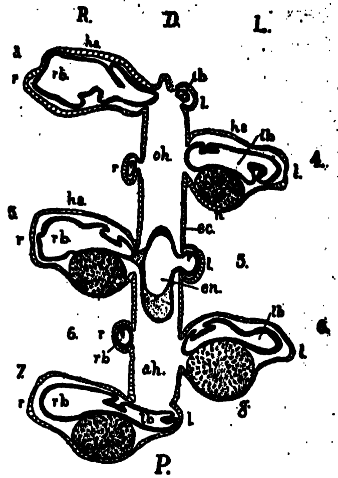


FIG. 13.

Nos. 3, 4, 5, 6 and 7 of Fig. 12, separated from each other by the imaginary elongation of the connecting tubes, in order to make room for the reference-letters. *P*, the proximal end of the stolon; *D*, its distal end; *R*, its right side; *L*, its left side; *r*, *r*, *r*, the right sides of the Salpæ; *l*, *l*, *l*, their left sides; *ec*, the ectodermal tube of the stolon (compare Fig. 10); *en*, the endodermal tube of the stolon; *oh*, oral hæmal tube of stolon; *ah*, ab-oral hæmal tube of stolon; *n*, ganglia and neural surfaces; *hs*, *hs*, hæmal surfaces; *rb*, right half of branchial chamber; *lb*, left half of branchial chamber.

In Nos. 8, 9 and 10 the ganglion (*n*) is towards the base of the stolon, and the right sides of all three towards the right side

of the stolon. Although the Salpæ form a double row here, as they do at a lower level, the sections in this plane show that this is only apparent, and is due to the necessity for finding room for the great ganglia of the closely packed series of animals. The figure shows that the series is in reality a single row of animals, with their neural surfaces towards the base of the stolon, and with one-half of the branchial sac and endostyle compressed into the space between two other animals, while the other side is free and enlarged. The reference-letters for the sections which are numbered 3–7 are on Fig. 13, which should be compared with Fig. 12.

In No. 7 the left half of the branchial sac (*lb*) and that of the endostyle are obviously on the left side (*L*) of the stolon, although the animal itself belongs to the series on the right side. Its ectoderm is continuous with the ectoderm of the bottom of the stolon, and its body-cavity communicates with the lower or ab-oral hæmal tube (*ah*) of the stolon, which is the same as the cavity shown in transverse section at *ah*, Fig. 10. In No. 6, a section of one of the animals on the left side, the body-cavity opens into the ab-oral hæmal tube (*ah*), the ectoderm is continuous with that of the stolon, and the right half of the endostyle and the right half of the branchial sac are separated from the corresponding portions on the left by the cavity of the stolon, which latter is therefore in the vertical plane between the letters *l* and *r* of No. 6, part of the body of the Salpa. No. 5 is a transverse section of the body at the level of the endodermal tube of the stolon, which is shown at *en* in Fig. 10. The tube is cut obliquely, so that the flat surfaces of its upper and lower walls are shown, as well as its central cavity. No. 6 shows that this cavity is here part of the branchial sac, and that one-half of the endostyle is on one side of it, and the other half on the other side. No. 4 communicates with the oral hæmal tube (*oh*) of the stolon in the same way that No. 6 communicates with the ab-oral one (*ah*), and in No. 3 the ectoderm of the body is continuous with that of the oral surface of the stolon, and the two divisions of the branchial sac are approaching, and in No. 2 they have met and united above the stolon.

If we now construct, in imagination, the body of a single chain-Salpa from the series of transverse sections, we shall find

that the cavity of the stolon is in reality part of the cavity of its body, and that the hæmal surface of the branchial sac of each Salpa gives rise between the folds of the endostyle to an endodermal tube which connects it with the neural surface of the branchial sac of the next one in the series, and that the Salpæ are not buds on the walls of the stolon, but segments of the stolon itself.

As the stolon is curved, it is difficult to get a long section in a single plane, but such a section at the level of No. 5 of Figs. 12 and 13 would give us the condition of things which is shown in Fig. 14. This figure is a diagram, formed by repetition of No.

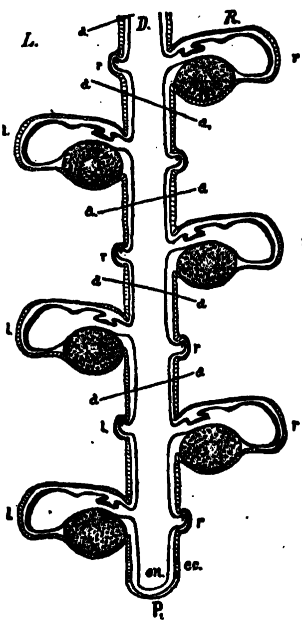


FIG. 14.

Diagrammatic horizontal section formed by repeating the sections shown in Figs. 14 and 12, No. 5. As the two sides of this figure are alike, I have reversed the lettering so as to bring the right side *R* of the stolon into the right side of the figure. *P*, the proximal end of the series; *D*, its distal end; *R*, the right side of the stolon; *r*, *r*, the right sides of the Salpæ; *L*, the left side of the stolon; *l*, *l*, the left sides of the Salpæ; *a—a*, *a—a*, the connecting tubes of the stolon; *ec*, its ectoderm; *en*, its endoderm.

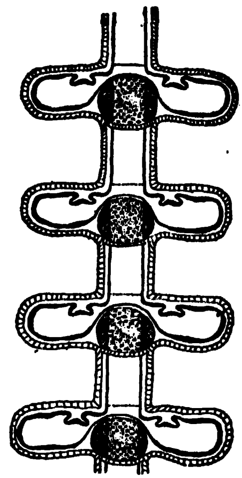


FIG. 15.

Oral view of Fig. 14 as it would appear if the ganglia retained their primitive positions in a single series above the tube of the stolon. An oral view of the diagram shown in Fig. 11 would be like Fig. 15.

5, of Fig. 13, and the endodermal tube (*en*) of the stolon is laid open longitudinally. The section from which Fig. 12 was drawn was mounted upon the slide with its oral surface below, and its right side on the left hand, but as Fig. 14 would be the same whether viewed from above or from below, I have reversed the reference-letters, and the figure is therefore supposed to lie with its oral surface towards the observer. The stolon is now seen to be made up of a series of unmodified connecting portions external to the Salpæ (*a*—*a*, *a*—*a*, *a*—*a*), and alternating with these a series of segments (*l*—*r*, *l*—*r*, *l*—*r*), which are converted into Salpæ. The bodies of the Salpæ form a single series, with the right sides (*r*) of all of them on the right side (*R*) of the stolon, and their ganglia towards its base. The ganglia do not, however, form a single series above the level of the stolon, as they are represented in Fig. 11, but a double series below its level, and, in accordance with this change in the positions of the ganglia, the branchial sacs have become compressed and distorted so that the right halves of one-half of them and the left halves of the others are very small. At an earlier stage the ganglia are above the stolon in a single series on the middle line, and if this position were permanent, the section which is shown in Fig. 14 would be like Fig. 15, which is also an oral view of the most advanced portion of Fig. 11.

The Salpa-chain is therefore a single series of animals like the Pyrosoma-stolon; the middle plane of the stolon is the same as those of the Salpæ: the right halves of all the bodies arise on the right half of the stolon, and their left halves on its left, and they are not formed by budding from its walls, but by the direct conversion of its tissues and cavities into those of the Salpa. The process is directly comparable, in every particular, with the published accounts of what occurs in Pyrosoma.

It will also be noticed that the egg-embryo or solitary Salpa is a member of the series, and that the only essential difference between it and the other members of the chain is its more rapid growth. The right half of each symmetrical organ arises on the right side of the stolon, and the cloaca is at first composed of two vesicles, which ultimately meet on the middle line and form a single cloaca directly comparable with the median atrium of Pyrosoma. The hearts and the digestive tracts, however, all

arise on the right side, although the left half of the endostyle and the left half of the branchial sac come from the left side of the stolon.

BALTIMORE, *March* 30, 1886.

EXPLANATION OF THE FIGURES.

FIG. 1, PAGE 455.

Diagram to show the normal or primitive positions of the Salpæ with reference to the long axis of the stolon. The black line is the middle line of the stolon; *P*, its proximal end; *D*, its distal end; the rectangles represent the bodies of the Salpæ; *r*, their right sides; *l*, their left sides; *h*, their hæmal surfaces, and *n*, their neural or cloacal surfaces.

FIG. 2, PAGE 455.

Diagram to represent Seeliger's view of the manner in which the Salpæ are formed upon the stolon. Reference-letters as in Fig. 1.

FIG. 3, PLATE XXVIII.

Diagram of the oral ends of the bodies of six of the Salpæ in a mature chain, to show their relative positions. *P*, proximal end of stolon; *D*, distal end of stolon; *a*, the series of Salpæ on the right side of the chain; *b*, the series of Salpæ on the left side of the chain; *n, n, n*, neural surface of the body of the chain-Salpa; *h, h, h*, hæmal surface; *r, r, r*, right side of Salpa; *l, l, l*, left side of Salpa.

FIG. 4, PLATE XXVIII.

Diagram to illustrate Seeliger's view of the origin of the bodies of the Salpæ on the stolon. *n, n, n*, neural surfaces; *h, h, h*, hæmal surfaces; *r, r, r*, right sides; *l, l, l*, left sides.

FIG. 5, PLATE XXVIII.

Diagram of a portion of a young stolon, to show the primitive positions of the Salpæ; letters as in Fig. 4.

FIG. 6, PLATE XXVIII.

Longitudinal section of the young stolon of Pyrosoma, from Kowalevsky. *ec*, ectoderm. *en*, endoderm; *n*, nerve-tube; *o*, ovary; *P*, parent; *b*, branchial sac of parent; *es*, endostyle of parent.

FIG. 7, PLATE XXVIII.

Transverse section of the young stolon of Pyrosoma, from Kowalevsky. *ec*, ectoderm. *en*, endoderm; *n*, nerve-tube; *c, c*, cloacal tubes; *o*, ovary.

FIG. 8, PLATE XXVIII.

Diagrammatic longitudinal section of an advanced Pyrosoma-stolon, compiled from the figures and descriptions given by Huxley and Kowalevsky. *P*, Parent; *I*, *II*, *III*, three segments of the stolon at successive stages of development; *b*, *b''*, *b'''*, branchial sacs; *c''*, *c'''*, cloacæ or median atria; *e*, excurrent aperture of cloaca; *ec*, ectoderm; *en*, endoderm; *es*, *es''*, *es'''*, endostyles; *n''*, *n'''*, ganglia; *o*, *o'*, *o''*, *o'''*, ovaries; *d'''*, stomach; *s*, stolon.

FIG. 9, PLATE XXVIII.

Longitudinal section of a young Salpa-stolon, from Seeliger's Plate I, Fig. 2. *ec*, ectoderm; *en*, endoderm; *n*, nerve-tube; *o*, ovary.

FIG. 10, PLATE XXVIII.

Transverse section of a young stolon of Salpa. *c*, *c*, cloacal tubes; *ec*, ectoderm; *en*, endoderm; *m*, mesodermal tubes; *n*, nerve-tube; *oh*, oral hæmal tube; *ah*, ab-oral hæmal tube; *o*, ovary.

FIG. 11, PLATE XXVIII.

Diagrammatic longitudinal section of an advanced Salpa-stolon, showing the constituent Salpæ in the positions which they would occupy were there no secondary changes of position during growth. *P*, parent or solitary Salpa; *I*, the youngest and most proximal chain; *II*, *II*, *II*, *II*, the series of ascidians which makes up the second chain; *III*, *III*, *III*, *III*, the series of ascidians which forms the oldest chain; *b''*, *b'''*, branchial sacs; *c''*, *c'''*, the cloacæ or median atria; *d''*, *d'''*, the digestive tracts; *ec*, ectoderm; *el*, eleoblasts; *h*, heart; *en*, endoderm; *es*, endostyle; *g*, gill; *n*, ganglia; *o''*, *o'''*, ova.

FIG. 12, PLATE XXIX.

A section through an advanced stolon transverse to the long axes of the bodies of the Salpæ, and inclined to the long axis of the stolon, so that the Salpæ 20 and 21, at the proximal end of the series, are cut through their nuclei and their eleoblasts, while those at the other end of the series (1 and 2) are cut through their oral ends. The section was mounted with the oral surface below, and the right half of the stolon *R* is therefore on the left side of the figure. *P*, proximal end; *D*, distal end; *L*, left and *R*, right side of stolon; *l*, left side and *r*, right side of each Salpa. If there were no displacement, the Salpæ would form a single series of 21 animals, as numbered, and the odd numbers have become crowded into the right side, and the even ones into the left by the pressure of adjacent Salpae.

a, anus; *c*, cloaca; *el*, eleoblast; *es*, endostyle; *g*, gill; *h*, heart; *i*, intestine; *lb*, left half of branchial sac; *rb*, right half of branchial sac; *l*, left side of Salpa; *r*, right side of Salpa; *o*, ovum; *s*, stomach; *m*, mouth; *n*, ganglia; *ns*, neural surface.

FIG. 13, PLATE XXIX.

Nos. 3, 4, 5, 6 and 7 of Fig. 12, separated from each other by the imaginary elongation of the connecting tubes, in order to make room for the reference-letters. *P*, the proximal end of the stolon; *D*, its distal end; *R*, its right side; *L*, its left side; *r*, *r*, *r*, the right sides of the Salpæ; *l*, *l*, *l*, their left sides; *ec*, the ectodermal tube of the stolon (compare Fig. 10); *en*, the endodermal tube of the stolon; *oh*, oral hæmal tube of stolon; *ah*, ab-oral hæmal tube of stolon; *n*, ganglia and neural surfaces; *hs*, *hs*, hæmal surfaces; *rb*, right half of branchial chamber; *lb*, left half of branchial chamber.

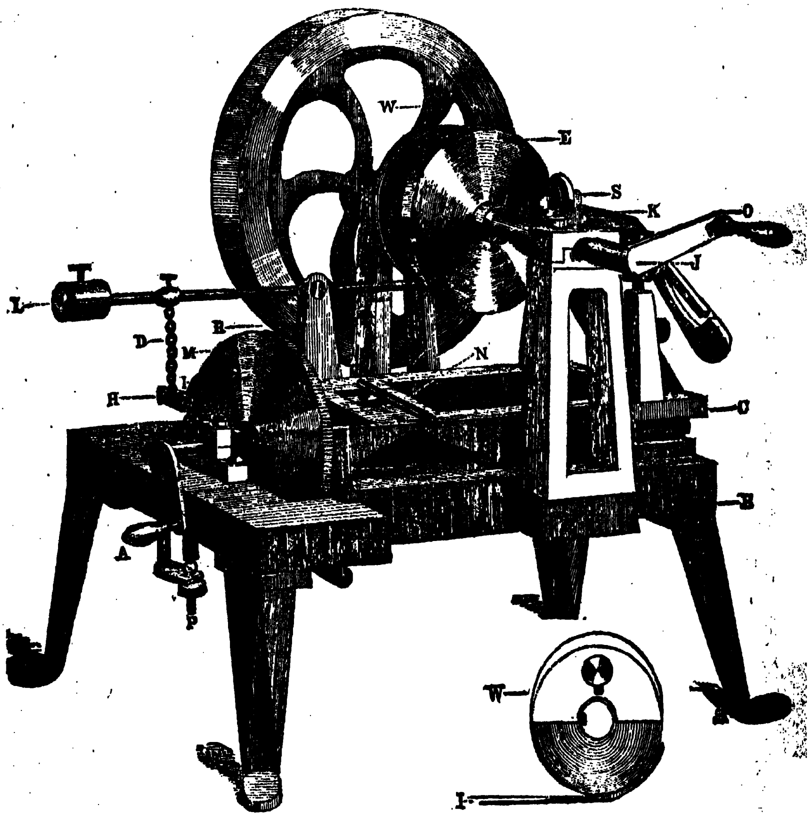
FIG. 14, PLATE XXIX.

Diagrammatic horizontal section formed by repeating the sections shown in Figs. 14 and 12, No. 5. As the two sides of this figure are alike, I have reversed the lettering so as to bring the right side *R* of the stolon into the right side of the figure. *P*, the proximal end of the series; *D*, its distal end; *R*, the right side of the stolon; *r*, *r*, the right sides of the Salpæ; *L*, the left side of the stolon; *l*, *l*, the left sides of the Salpæ; *a* — *a*, *a* — *a*, the connecting tubes of the stolon; *ec*, its ectoderm; *en*, its endoderm.

FIG. 15, PLATE XXIX.

Oral view of Fig. 14 as it would appear if the ganglia retained their primitive positions in a single series above the tube of the stolon. An oral view of the diagram shown in Fig. 11 would be like Fig. 15.

REVOLVING AUTOMATIC MICROTOME.



The microtome represented in the cut above is the invention of Adam Pfeifer, mechanic and instrument-maker to the Biological Laboratory of the Johns Hopkins University.

The machine is designed to save time and labor in the preparation of series of sections, and to attain at the same time the greatest uniformity in the thickness of the sections.

The mechanism is very simple. The frame (*Fig. B*) contains a horizontal screw beneath the sliding carriage (*C*). The carriage carries the knife (*K*). This carriage is moved forward by turning of screw. Two arms of the frame support the axis (*J*) of the revolving wheel (*E*), to which the imbedded object is attached. The knife (*K*) is clamped in an upright position on the arms rising from the sliding carriage, so that the edge of the knife is in the same horizontal plane with the centre of the axis (*J*). Thus, as the sliding carriage is moved by the screw, so the knife is moved to or from the revolving object. The carriage slides by means of grooves on raised tracks of the frame, and is not directly connected with the screw, but is simply pushed by nut (*N*). This arrangement makes it impossible that any slight eccentricity of the screw should cause a jolting of the carriage.

The head of screw is a solid wheel (*M*) at the end of frame, and has 250 ratchet-teeth on its circumference. The screw has 20 threads to the inch (=.025 m.). The knife, therefore, is moved an inch by 20 revolutions of the screw; and as there are 250 teeth to the revolution, each tooth represents $\frac{1}{20 \times 250}$ $= \frac{1}{5000}$ inch (.005 mm.).

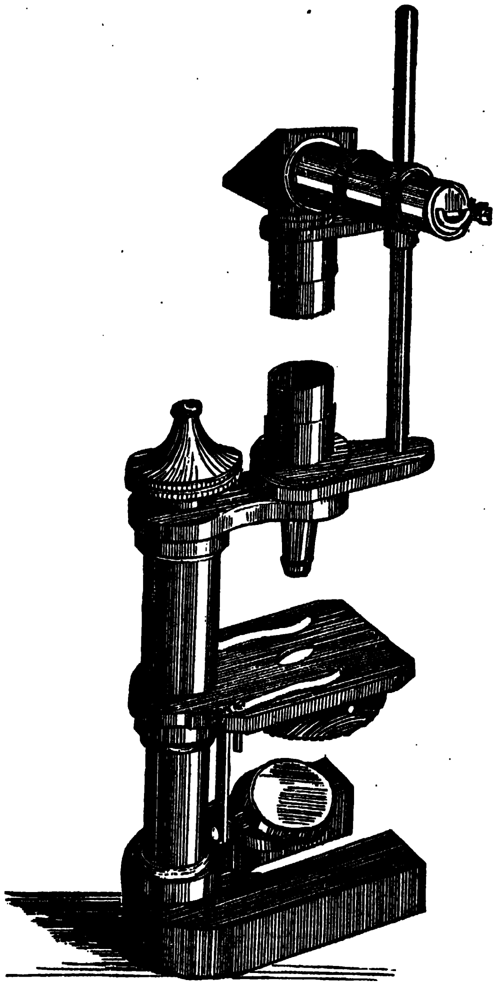
The handle (*O*) turns the axis (*J*), to which is attached the wheel (*E*). This wheel is four inches in diameter, and to it is fastened the clamp which holds the object to be cut. The axis also carries a fly-wheel and an adjustable eccentric wheel (*W*), which is figured apart in a corner of the illustration. This eccentric moves a lever (*L*), the long arm of which is connected with the small chain (*D*). The chain lifts a small lever (*H*), which works by means of a catch (*I*) on the teeth of the screw-head, causing the screw to revolve. The small lever is steadied and pulled back to its place by a spiral spring (*P*), while another spring catch underneath the frame prevents the ratchet-wheel from turning back. By properly adjusting the eccentric wheel the levers may be made to act so that the catch (*I*) will take any desired number of teeth by every revolution of the object. The knife moves only during that part of the revolution when the object is not in contact with the knife. The ribbon of sections slides downward from the knife and is caught on a piece of paper placed upon the table.

The wheel holding the object, as well as the razor, can be moved so that almost all parts of the edge of the razor can be used.

The frame bed of the microtome is made of iron, the screw of steel, and all the rest is brass. Any ordinary microtome knife or razor may be used.

The machine has been in use here for a year and gives the greatest satisfaction. It can be used with great rapidity, but so far the best results have been obtained at a rate of not over 100 sections to the minute. The only possible error in a revolving microtome of this kind is theoretical—namely, that owing to the circular motion of the object, each section is part of a hollow cylinder. But in reality, with objects of ordinary size, this error is not apparent, and even under a high magnifying power there is no perceptible difference between sections cut by this microtome and those cut by ordinary slide microtomes.

EMBRYOGRAPH FOR USE WITH ZEISS MICROSCOPES.



This piece of apparatus, which is the work of Adam Pfeifer, the instrument-maker of the Biological Laboratory of the Johns Hopkins University, renders the Zeiss-Oberhausen camera avail-

able for drawing objects under very low magnifying powers. It consists, first, of a collar fitted to the arm of the microscope, and furnished with a short draw-tube, which can be placed with the objective either above or below the arm; and, second, of a vertical rod, supported on an arm which is clamped under the collar of the draw-tube, and carries a second movable arm resting in a collar to support the camera. This arm is held in place by a thumb-screw, and it may be set at any point on the vertical rod. When the Zeiss *a. a.* objective is used, and the camera is lowered as much as possible, an image magnified about three diameters is projected on to the paper, and any amplification greater than three diameters may be obtained by varying the height of the camera, and by the use of the higher objectives.

ON THE INFLUENCE OF ALCOHOLS ON THE CONVERSION OF STARCH BY DIASTASE. By J. R. DUGGAN, M. D., Ph. D., Professor of Chemistry in Wake Forest College, N. C.; Late Fellow of the Johns Hopkins University.

On account of their marked action on living organisms, and simple chemical structure, the alcohols are well adapted for studying the relation of chemical constitution to physiological action, and have been used for this purpose by several observers. The conditions of even the simplest life-processes are so complicated that physiological action cannot be determined with accuracy by direct experiment; and those made on alcohols have as yet shown only that, in general, their activity increases with the number of carbon atoms they contain. It occurred to me that this activity might show itself by producing a corresponding retardation in the conversion of starch by diastase. Of course it is not necessarily true that the action of a substance on a soluble ferment is proportionate to its action on living organisms, or that these are in any way related, but it is natural to suppose that the physiological action of an alcohol is due to some definite chemical property or properties; and, this being true, it seems not improbable that this same property should retard diastatic action, so that the amount of any alcohol required to bring about a certain degree of retardation could be taken as a measure of its physiological strength.[1] If it could be shown that this is true, it would be a very great gain, for results could then be obtained having the accuracy of a chemical analysis.

The following table shows the percentage by weight of each of a number of alcohols, required to retard the action of diastase one-half:

[1] The term "strength" is used here to avoid circumlocution. I think it will be seen later in what sense it is used.

TABLE I.

Class.	Name.	Formula.	Per cent. required.
Primary	Methyl,	HCH_2OH	9.18
Primary	Ethyl,	CH_3CH_2OH	6.82
Primary	Propyl,	$C_2H_5CH_2OH$	3.48
Primary	Butyl, iso.,	$(CH_3)_2CHCH_2OH$	2.50
Primary	Amyl from fermentation,		1.16
Secondary,	Propyl,	$(CH_3)_2CHOH$	4.48
Tertiary,	Butyl,	$(CH_3)_3COH$	7.96
Unsaturated,	Allyl,	$C_2H_3CH_2OH$	4.96
Diatomic,	Glycol,	$C_2H_4(OH)_2$	about 25.00
Triatomic,	Glycerol,	$C_3H_5(OH)_3$	" 45.00
Hexatomic,	Mannitol,	$C_6H_8(OH)_6$	no action in 10 p. c. sol.

The amyl alcohol used was that obtained by fermentation, which produces a mixture of two or more primary alcohols. The one having the formula $C_2H_5.CH_3.CH_2OH$ is present in the greatest proportion. On account of the large amounts of glycol and glycerol required, and some doubt about their purity, the figures given cannot be considered accurate. In the case of some of the other alcohols, the quantity at my command was such that I could not make myself quite sure about their purity. As will be seen later, the presence of an extremely minute quantity of some acid may introduce a considerable error into the result. I hope to repeat the. experiments with even greater precautions than could be taken with these, but I do not think that any noticeable error will be found in the above figures.

The following method was used in all of the experiments: The starch-paste was made by adding enough boiling water to 25 grams of the best arrowroot starch to make one litre. For the control experiment 98 cc. of this were used, and for the others 49 cc., which was made up to 98 cc. by the addition of the alcohol and water. The required amount of alcohol was found by using first a certain amount, and increasing or decreasing this according as the action was below or above 50 per cent. of that in the control flask. As within certain limits the percentage of retardation is nearly proportional to the quantity of alcohol present, the required amount could usually be calculated after a few experiments; but another determination was always made to prove the correctness of this. The flasks containing the starch-

paste were all placed in a water-bath, which was kept at 55° C., and when they had attained a uniform temperature 2 cc. of a solution of diastase were added to each. At the end of half an hour the reaction was stopped by the addition of a few drops of a strong solution of caustic soda, and the amount of sugar formed in each determined by Fehling's solution volumetrically. Although the amount of starch present, provided there is a considerable excess, does not affect to any great extent the action of the ferment, by using 98 cc. of paste in the control and 49 cc. in the others, the amount of starch present is, in each case, proportionate to that of the sugar formed.[1]

Although the number of alcohols examined is not large, it seems safe to draw two conclusions concerning their action on diastase:

1st. Their activity increases on the addition of each CH_2 group, as is shown by the five members of the primary series.

2d. Their activity decreases on the addition of each OH group, as is seen from the fact that glycol and glycerol have but little action, especially the latter, while mannitol has too little, if any, to be determined.

It would also appear that the secondary act less than the primary, and the tertiary less than either; but, as only one secondary and one tertiary have been examined, this point cannot yet be considered as established. The result obtained from the single member of the ethylene or unsaturated series points to the conclusion that a decrease in the number of hydrogen atoms causes a decrease in activity; but here again it is not safe to form a conclusion. A change in that portion of the molecule not included in what is known as the alcohol residue probably influences the action of the alcohol; but this point must be left for further experiments to decide.

The principal object of this investigation being to find if the results given in the above table have any connection with the physiological activity of the corresponding alcohols, it is necessary to consider all that has been done in this line in the way of experiments on living organisms.

[1] For further details on this and other points connected with the determination of diastatic action, a paper by the author in the Amer. Chem. Jour., Vol. VII, No. 3, may be consulted.

Dujardin-Beaumetz and Audigé[1] have studied the toxic action of the fermentation alcohols by determining the amount of each necessary to kill dogs in twenty-four hours. I think that every one will admit that this method can give, under the best conditions, only the roughest approximation; and, as would be expected, their results show considerable variation. The following table gives the average number of grams per kilo. of the dog's weight that produced a fatal effect when injected hypodermically, undiluted:

TABLE II.

Ethyl,	7.09 grams.
Propyl,	4.32 "
Butyl,	2.15 "
Amyl,	2.02 "

Methyl alcohol, although not produced by fermentation, was added to this list, and found to act about the same as ethyl, seven grams being required. As the alcohols from fermentation are usually mixtures of several isomers, and the authors do not state if any attempts at separation were made, these results lose much of their value as approximate measures of physiological action. The authors attempt to prove that the four fermentation alcohols act in the proportion $1:\frac{1}{2}:\frac{1}{3}:\frac{1}{4}$, but the evidence is by no means sufficient for this.

More recently Ringer[2] has studied the action of alcohols on the frog's heart, and he obtains the following figures as representing the relative amount of each required to produce a certain effect:

TABLE III.

Methyl,	205	Butyl,	17
Ethyl,	114	Tertiary Butyl,	40.5
Propyl	59	Amyl,	6.6
Isopropyl,	44.5		

The details of the method by which these figures were obtained are not given in a way that allows one to judge of their accuracy; but what, for theoretical reasons, seems to be an error in one case will be noticed later.

[1] Compt. Rend. de l'Acad. 88, 80. [2] Practitioner, 30, 339.

Some time ago I endeavored to find the amount of each of the first three primary alcohols that is required to prevent, for a given time, the development of bacteria in a standard solution of beef peptones.[1] As near as could be determined, the percentage required was: of methyl, 3; of ethyl, 5, and of propyl, 2. I regret that I have not yet been able to include a larger number of alcohols in this series.

It will be seen that further study is necessary to show if the action of alcohols on diastase bears any relation to their action on living organisms; for while all of the observations show that in each case we have increased activity in the higher members of the series, it cannot be said whether the differences observed are actual, or are due to errors of methods and observation. It is certain that in some of the results given these are large enough to make this possible.

An important point in studying this question is: Do all alcohols act in the same proportion on all living organisms? In the experiments of Dujardin-Beaumetz and Audigé on dogs, and more especially in my own on bacteria, ethyl alcohol causes a break in the series by showing less action than methyl. No such result is seen in Ringer's experiments on the frog's heart, or in my own on diastase.[2] Now, as ethyl alcohol is exactly similar in its constitution to the other members of the normal primary series, one is at a loss to account for this, unless we suppose that organisms can establish a tolerance of one alcohol, and retain their sensitiveness to others. My experiments, which showed very markedly this diminished action of ethyl alcohol, were made on the bacteria of putrefaction, and in solutions undergoing decomposition this alcohol is probably always present, and frequently in noticeable quantities, so that such bacteria evidently have had opportunity to become tolerant of its presence. It is much more difficult to show that dogs should be tolerant of its action, although it is probable that they get a little in their food, such as is in bread from fermentation; and it is possible that it

[1] Amer. Chem. Jour. VII, 62.

[2] Researches made in the Johns Hopkins Biological Laboratory last year by Dr. J. C. Hemmeter, but not yet published, show that so far as the isolated heart of the dog is concerned, ethyl alcohol also causes a break in the series; being less hurtful than methyl.—Ed.

is formed in their alimentary canal from fermentation, or from oxidation of saccharine matter. It should also be noticed that in the experiments on dogs, ethyl alcohol comes much nearer to what we would suppose, from chemical reasons, is its proper place in the series, so that if this variation is due to tolerance, it is much less marked with dogs than with bacteria of putrefaction. In the human species we should expect very considerable tolerance of ethyl alcohol to show itself; and although there are no direct experiments on this point, it is well known that both lower and higher members of the series are considered poisonous in quantities in which ethyl alcohol produces no apparent effect. In the case of frogs, where there is no reason to suppose that they are subjected to the constant influence of any alcohol, we find that the series is regular, ethyl being intermediate in its action between methyl and propyl. I am very well aware that the facts here noticed are not sufficient to base a conclusion on, but they seem to be of enough interest to warrant their consideration; for if it be true that an organism can become tolerant of one member of a series of compounds and retain its sensitiveness to the others, this will have an important bearing on investigations intended to establish the relation between chemical constitution and physiological action. Some experiments that I have made on the action of acids on bacteria tend to establish this point, but they are not yet ready for publication. I hope soon to try the action of several alcohols on yeast, and I think that I shall find that here ethyl alcohol shows, proportionately, even less action than on bacteria. Ringer's experiments show that tertiary alcohols act on the frog's heart much less than primary or secondary, just as in the case of diastase; but he found that the secondary act rather more than the primary, while the reverse is true with diastase. I am inclined to think, however, that his results on this point are wrong, not because they differ with my own, but because for chemical reasons we should expect the secondary to be intermediate between the primary and the tertiary.

In considering this investigation, the question suggests itself as to whether or not there is in all alcohols some chemical property, which we may term "alcoholicity," just as with acids we usually have acidity, and with bases alkalinity. I will give a few

experiments that I have made on this point, although they are as yet very incomplete. If, to the alcohol used to retard diastatic action 50 per cent., a very little acid is added, the retardation becomes very slight, as is shown by the following table:

TABLE IV.

	Amount of sugar formed.
1. Diastase and starch-paste alone (100 cc.),	0.480 gram.
2. With 8 cc. ethyl alcohol,	0.248 "
3. With 0.4 mgr. hydrochloric acid,	0.460 "
4. With 8 cc. alcohol and 0.4 mgr. HCl,	0.427 "

It is seen from this that if the same amount of acid be used alone, it has a slight retarding effect, so that it cannot be supposed that the acid acts in Exp. 4 by stimulating in some way the action of the ferment; it must act by neutralizing the influence of the alcohol. Acetic acid gave similar results, except that a larger quantity, since it is a weaker acid, was required. It is well known that most alcohols, especially those of the primary series, have very slight alkaline properties, as is shown by the fact that they retard the inversion of cane sugar by acids, and it seems that a great part of their action on diastase, is due to their alkalinity.[1] This ferment is so delicate an indicator, that I hope to be able by the use of it to measure the alkalinity of the various alcohols.

The secondary and tertiary alcohols approach the acid phenols in their constitution, and are therefore less alkaline. This corresponds with their action on diastase. It is interesting to note also that the action of organic acids on diastase is less with higher members of a series, while increasing their basicity increases their activity. The alcohols, on the contrary, show the greatest action with higher members of a series, while increasing their atomicity decreases their activity.

As to how far the physiological action of alcohols is due to

[1] The action of acids and bases on diastase, and the use of this ferment as an indicator, are discussed in an article by the author in the Amer. Chem. Jour. VIII, p. 211, and the reader must be referred to this for the meaning of the terms acidity and alkalinity, as used here. They have no connection with the property of some bodies to combine with bases to form salts, or of others to combine with acids, or what is properly known as basicity.

their alkalinity, cannot at present be surmised, but I am inclined to think that this plays an important part in it. If this is true, it may be asked why ordinary alkalies do not act in a similar manner. The distinction between alkalinity and basicity must be kept clearly in mind here. If ordinary alkalies are taken into the stomach, they are very quickly neutralized by combination with an acid; but in the case of alcohol we have an alkaline body that probably cannot be neutralized by the acids of the stomach, for it is only at high temperatures that alcohol will combine with dilute acids to form esters (salts). In this way, it will be seen, alcohol may act through its alkalinity, while alkaline bases show no such action. It should be mentioned that this, as well as other points discussed in this paper, are put forward more as suggestions towards future work than well-developed theories.

ON THE ACTION OF CERTAIN SALTS UPON THE ARTERIES.

By FREDERIC S. LEE, M. A., Ph. D., Instructor in Physiology in St. Lawrence University, Canton, N. Y.; Late Fellow of the Johns Hopkins University.

Physiologists now generally acknowledge that arterial tonicity may result from two sorts of influences, those of central and those of peripheral origin. As regards the latter sort, Bidder in 1866 ascribed to peripheral ganglia the power of causing arterial constriction; but the claim of Bernstein, that it is most reasonable to suppose the local tone to originate in the arterial muscle-cells themselves, has been recently gaining ground. Yet, if we accept Bernstein's view, we must still look for the cause of the contraction of the muscle-cells, and for the variations in the amount of such contraction which are known to take place normally in the body. Up to the present time but one theory, I think, has been advanced to explain these phenomena—that proposed by Gaskell[1] in 1880.

Gaskell found that the addition of a small quantity of a solution of sodic hydrate to a neutral fluid circulating through the arteries of a frog was followed by a constriction of the vessels to a degree varying with the amount of the drug present. He further found that lactic acid introduced into the neutral circulating fluid acted in an opposite manner, putting the arteries into extreme dilation. Moreover, the constriction brought about by sodic hydrate was with the acid quickly replaced by dilation; and the dilation produced by lactic acid was with the hydrate quickly replaced by constriction. From these facts Gaskell drew the general conclusion that alkalies cause constriction and acids cause dilation of the arteries, and he proposed to account for the ever-present arterial tonicity by supposing the alkaline juices bathing the walls of the arteries to exert their constricting action upon the muscle-cells. The production of an acid by tissue-activity, as *e. g.* the contraction of a muscle, would diminish the alkalinity of the fluids, and hence diminish

arterial tonicity. Variations in the latter would thus be explained by variations in the degree of alkalinity of the surrounding fluids. Gaskell extended his theory to the explanation of cardiac tonicity, since sodic hydrate and lactic acid were found to have upon the heart effects similar to those exerted upon the arteries—*i. e.* the former brought about a standstill of the heart in systole, the latter a standstill in diastole.

The founding of so radical a theory upon such apparently insufficient experimental grounds—for the author had employed in his work but one alkali, and that not one which is present in the body—was noticed by Ringer and Buxton,[2] and they were led to investigate the question more fully by experiment. They studied the heart only, obtaining with sodic hydrate the same results which Gaskell had obtained. Turning then to the normal alkalies of the blood, they found that sodic bicarbonate gave a similar stoppage of the heart in systole. With sodic phosphate alone, "a possible source of alkalinity other than the bicarbonate," but little increase in cardiac tonicity was observed. But they further found that potassium chloride, also a normal constituent of the bood, when introduced into the neutral circulating fluid, acted quite similarly to lactic acid, putting the heart into a state of extreme relaxation. After the production of a systolic condition by sodic hydrate or sodic bicarbonate, the addition of a small quantity of potassium chloride completely counteracted the effect of the alkali, "the tonicity of the heart-muscles giving place to relaxation." They did not extend their researches to the arteries; but Ringer and Sainsbury[3] had previously shown that sodic bicarbonate by itself causes arterial constriction, yet this condition does not appear in the presence of potassium chloride. Ringer and Buxton hence claimed that neither cardiac nor arterial tonicity could be explained as due to the direct action of the alkaline juices upon the muscle-cells, since the constant presence of potassium chloride would prevent the sodic bicarbonate from exerting its characteristic action.

The antagonism of the views of Gaskell and of Ringer and Buxton, and the lack in the latter's work of experimental evidence as to the arteries, have induced me to test for those vessels the validity of the objections to Gaskell's theory.

My method of work has been essentially the same as that employed by Stevens and myself[4] in determining the action of fibrin ferment upon the arteries, and is described in full in our paper upon that subject. In all of my experiments terrapins were employed, and the brain and spinal cord were completely destroyed at the beginning of the operation. The anterior portion of the plastron was then removed, exposing the heart and the great vessels; the heart was cut out and the inflow cannulas were inserted into the left systemic aorta and into the *bulbus arteriosus* just before the right systemic aorta was given off; the pulmonary arteries were ligated, together with all arteries supplying the head and fore legs, since the rupture of the vessels by the destruction of the brain and spinal cord would not have allowed a circulation through those parts; a pressure cannula was inserted into the stump of the right brachial artery; a large outflow cannula was placed upright in the *sinus venosus*, and the animal was then ready for the experiment. The circulating fluid was a 0.75 per cent. solution of NaCl in distilled water, and was supplied from a Marriotte's flask. Between the flask and the animal's body was placed, in connection with the inflow tubing, the automatic lever described by Stevens and myself, its function being to force into the arteries of the animal in an intermittent stream the circulating fluid coming from the flask. The venous pressure of this artificial heart—*i. e.* the height of the lower end of the inflow air tube of the Marriotte's flask above the lever—was 19 cm., and the animal was placed on a level with the lever. A circulation was thus possible through the hind limbs and abdominal viscera; and the circulating liquid, returning to the venous sinus, was caught, as it came through the outflow tube, and measured at intervals of three minutes. Arterial pressure was registered upon a revolving drum. A drug, the action of which was to be tested, was dissolved in normal salt solution and supplied to the arteries from a second flask standing beside the one containing the neutral circulating fluid. I have investigated the actions of sodic hydrate, sodic bicarbonate, sodic phosphate, lactic acid and potassium chloride. The sodic bicarbonate was manufactured by Merck, of Darmstadt. The sodic phosphate was of the same manufacture and was purified and recrystallized before using. The potassium chloride was supplied by Eimer &

Amend, of New York, and was also carefully recrystallized, to exclude all possibility of the presence of any impurity.

I found sodic hydrate to be the most powerful of all the drugs used, as regards the constriction of the arteries, a solution of strength 1 to 20,000 (*i. e.* 1 part drug to 20,000 parts salt solution) causing a rapid diminution in the outflow, accompanied by a great rise of arterial pressure.

Solutions of sodic bicarbonate, 1 to 2500, caused constriction, and stronger solutions produced much more marked effects upon the pressure and outflow.

Solutions of sodic phosphate, 1 to 500, gave slight constriction; solutions of strength 1 to 250 narrowed the arteries very considerably, in one case diminishing the outflow from 30.4 cc. to 5.2 cc., and increasing the pressure from 18 mm. to 24 mm. Hg. These results with sodic phosphate are quite opposed to those obtained from the heart by Ringer and Buxton. My experiments with the drug have been numerous, and in no case has it failed to produce constriction.

Lactic acid in proportion 1 to 10,000 gave dilation. Contrary to the testimony of Gaskell, this dilation could be recovered from by normal salt solution alone.

Potassium chloride, 1 to 10,000, produced, as Ringer and Buxton claim, dilation, the action being distinct and undoubted, though in some cases not persisting long, even when KCl was kept circulating. The action of the drug seems, however, to have been somewhat exaggerated by Ringer and Buxton, for I have found that dilation produced by a comparatively strong solution of KCl (1 to 2000) is at once set aside and constriction brought about by the addition of $NaHCO_3$ in proportion 1 to 1000.

Ringer and Buxton lay great stress upon the fact that a condition of increased tonicity produced by a drug, as *e. g.* sodic bicarbonate, may be replaced by an atonic state upon the addition to the circulating fluid of a sufficient quantity of potassium chloride—a proposition which no one can deny. But to conclude from this, as they do, that the alkaline salts of the blood do not maintain arterial tonicity, is unjustifiable. To reproduce in an experiment "the physiological conditions which are present in the blood," as they desire to do, it is necessary to introduce into the circulating liquid *at the same time* both $NaHCO_3$ and KCl,

and these in proportions such as they exist in the blood itself. So far as it appears from the paper of Ringer and Buxton, they have not done this. In attempting it we are, of course, at once hampered by the fact that absolute knowledge regarding the salts of the plasma is so far wanting. The blood of the terrapin has, I believe, never been completely analyzed. Of mammalian blood, the analyses of Schmidt and Sertoli are probably reliable, and in experiments which I have made upon the point in question I have used their results. According to Schmidt, plasma contains of Na_2O 0.1532 per cent., which, computed as sodic bicarbonate, its probable compound in the blood, equals 0.2075 per cent. Sertoli finds 0.116 per cent. of Na_2O in serum, equivalent to 0.157 per cent. of $NaHCO_3$. The latter does not mention potassium chloride in his table, and I have hence employed with each proportion of $NaHCO_3$ the proportion of KCl given by Schmidt—viz.: 0.0359 per cent. The following are some of the results:

Experiment 24.

Time of obs.	Temp of animal in deg. C.	Arterial pressure in mm. Hg.	Total venous outflow in cc.	Remarks.
5.51	15.9	18	14.6	0.75 per cent. solution of NaCl circulating.
.54	...	18.1	14	Solution of KCl on, 1 to 10,000. It must be borne in mind that, owing to the considerable length of tubing connecting the feeding-flask with the animal, which was necessitated by the condition of the experiments, several minutes may elapse, after turning on a solution, before it begins to enter the animal's arteries; so, too, it may continue to flow for some time after it has been shut off. Naturally these intervals are longer where the venous outflow is small.
.57	...	18.1	13.9	
6.00	...	18	13	
.03	...	18	14.2	
.06	...	17.9	15.5	
.09	...	17.9	16	
.12	...	17.8	16.5	
.15	...	17.7	21	At 6.14 solution of $NaHCO_3$ (1 to 2000) added to the circulating liquid.

Time of obs.	Temp. of animal in deg. C.	Arterial pressure in mm. Hg.	Total venous outflow in cc.	Remarks.
.18	16.2	17.9	19.9	
.21	...	18.4	16.8	
.24	...	18.6	12.1	
.27	...	18.7	9.8	
.30	...	19.3	8.5	Experiment ended.

In the above table it will be noticed that the ratio of sodic bicarbonate to potassic chloride in the circulating liquid after 6.14 was nearly the same as that of sodic bicarbonate in Sertoli's analysis to potassic chloride in Schmidt's.

Experiment 32.

Time of obs.	Temp. of animal in deg. C.	Arterial pressure in mm. Hg.	Total venous outflow in cc.	Remarks.
4.43	...	18.9	15.6	0.75 per cent. solution of NaCl circulating.
.46	...	18.7	15.5	
.49	...	18.7	15.8	
.52	...	18.4	19.4	On 0.157 per cent. $NaHCO_3$ (Sertoli) + 0.0359 per cent. KCl (Schmidt).
.55	...	19	18	
.58	...	18.8	19.8	
5.01	...	19	17.8	At 5.00 off $NaHCO_3$+KCl. On NaCl.
.04	...	23.1	11	
.07	16.8	28	8	
.10	...	25.4	6.8	
.13	...	23.2	6	
.16	...	22.9	7.2	
.19	...	21.5	6.8	
.22	...	20.3	7.9	
.25	...	19.2	8	
.28	...	19.3	9	
.31	...	19.1	10	
.34	...	19.2	10.4	
.37	...	19	9.8	
.40	...	19.1	10	
.43	...	19.1	9.7	
.46	..	19.1	8.8	
.49	...	18.9	10.5	
.52	...	...	10.3	
.55	16.1	18.6	12.8	
.58	...	18.2	13.4	
6.01	...	18.1	13.9	
.04	...	18.1	12.9	

Time of obs.	Temp. of animal in deg. C.	Arterial pressure in mm. Hg.	Total venous outflow in cc.	Remarks.
.07	...	18	14	
.10	...	18.5	13	
.13	...	18.3	19	
.16	...	18.4	14.9	
.19	...	18.5	14.8	
.22	...	18.7	13.4	
.25	...	18.4	14	
.28	...	18.9	15	At 6.26 on 0.2075 per cent. $NaHCO_3$ (Schmidt) + 0.0359 per cent. KCl (Schmidt).
.31	...	18.7	16.8	
.34	...	18.5	24.5	Off $NaHCO_3$ + KCl. On NaCl.
.37	...	19.1	24	
.40	...	21.1	12.5	
.43	...	21.4	9	Experiment ended.

Experiment 34.

Time of obs.	Temp. of animal in deg. C.	Arterial pressure in mm. Hg.	Total venous outflow in cc.	Remarks.
3.39	...	18.8	17	0.75 per cent. solution of NaCl circulating.
.42	...	18.9	23	
.45	17.9	18.9	29	On 0.2075 per cent. $NaHCO_3$ (Schmidt) + 0.0359 per cent. KCl (Schmidt).
.48	...	18.7	32	
.50	...	19	24.6	Off $NaHCO_3$ + KCl. On NaCl.
.53	...	22	26	
.56	...	28.4	12	
.59	...	25.4	7.9	
4.02	...	24.4	6	
.05	17.9	22.6	4.8	
.08	...	22.1	5	
.11	...	20.4	5.7	
.14	...	20.2	6	
.17	...	20	5.4	
.20	...	19.8	5.5	
.23	...	19.8	7.2	On $NaHCO_3$ (Schmidt) + KCl (Schmidt).
.26	...	19.5	7	
.29	...	19.4	16.4	
.32	...	19.6	24	
.35	...	20.1	16	
.38	...	20.7	8	
.41	...	21.7	6.5	

Time of obs.	Temp. of animal in deg. C.	Arterial pressure in mm. Hg.	Total venous outflow in cc.	Remarks.
.44	...	20.9	4.3	Off $NaHCO_3$ + KCl. On NaCl.
.47	...	...	4.8	
.51	...	30.8	9.5	Greater outflow here is due to longer time of observation.
.54	17.9	31.6	5.1	
.58	...	25.5	6.8	Ditto.
5.01	...	25.5	4.3	
.04	...	21.8	7	
.07	...	20.5	4.9	
.10	...	19.8	5.5	
.13	...	19.9	7.9	

Experiment 35.

Time of obs.	Temp. of animal in deg. C.	Arterial pressure in mm. Hg.	Total venous outflow in cc.	Remarks.
3.21	21.3	22.7	6.1	0.75 per cent. solution of NaCl circulating.
.24	...	21.9	5.2	
.27	...	21.9	5.1	On 0.2075 per cent. $NaHCO_3$ (Schmidt) + 0.0359 per cent. KCl (Schmidt).
.30	...	21.8	6.4	
.33	...	21.4	7.3	
.36	...	21.5	7.2	
.39	...	23	5.7	
.42	...	23.5	4.4	
.45	...	25.9	3.8	
.48	...	26.4	4.8	
.51	...	29.9	3.8	
.54	...	28.9	4.1	
.57	...	30.5	3.6	
4.00	...	28.9	4.5	Off $NaHCO_3$ + KCl. On NaCl.
.03	...	30.5	4.3	
.06	...	33.7	3.7	
.09	...	27.6	3.9	
.12	...	37.5	4.3	
.15	...	35.8	2.5	
.18	...	32.8	2.1	
.21	...	31.7	3	
.24	...	28.4	3.2	
.27	...	25.9	4.4	
.30	...	24.7	3.8	
.33	...	23	3.9	
.36	...	22.8	5	
.39	...	21.9	3.6	

Time of obs.	Temp. of animal in deg. C.	Arterial pressure in mm. Hg.	Total venous outflow in cc.	Remarks.
.42	...	21.8	5.7	
.45	...	22.3	5.4	
.48	...	21.7	5.1	
.51	...	21.7	5.2	On $NaHCO_3$ (Schmidt) + KCl (Schmidt).
.54	...	22	5.5	
.57	...	21.7	5.7	
5.00	21.3	21.7	5.6	
.03	...	21.7	5.3	
.06	...	23.8	4.8	
.09	...	24.4	4.3	
.12	...	25.4	4.5	Off $NaHCO_3$ + KCl. On NaCl.
.15	...	25.8	4.4	
.18	...	25.9	4.3	
.21	...	25.5	4.2	
.24	...	33.5	4.3	
.27	21.3	30.2	3.4	Experiment ended.

In all these cases we see that the ultimate effect of the mixture of $NaHCO_3$ and KCl is constriction of the arteries, manifested by a rise of pressure and a decrease in venous outflow. And this appears in those cases where Sertoli's amount of the former drug was used, as well as in those where Schmidt's results were employed. The constriction is somewhat slow to appear, and sometimes seems to be preceded by a slight and momentary dilation, manifested only by an increased outflow, as if the KCl first affected the muscle-cells, but was soon overcome by the more powerful but slowly acting $NaHCO_3$. The tables illustrate one other phenomenon frequently met with—*i. e.* the great difficulty experienced in bringing back the vessels to their former condition by the use of normal salt solution. After the alkaline solution is turned off, the constriction usually continues to rise and the pulse-curve often becomes irregular, now rising, now falling. This continues for several minutes, but the NaCl gradually forces its way into the vessels and washes out the alkali, and the arteries finally succumb and return to their former dilation.

From the results of my experiments upon the effects of sodic bicarbonate, sodic phosphate and potassic chloride, it would then appear that the quantity of the chloride normally present in the blood is insufficient to prevent the constricting action of the alkaline salts upon the arteries; such action is due

chiefly to the sodic bicarbonate, but the tendency of the sodic phosphate is in the same direction. But the effect of these two salts alone, in the proportions in which they exist in the blood, would be enormous, and a serious hindrance to circulation. The slight dilating action of the potassic chloride would hence seem necessary to modify and tone down the constriction, and its presence in the blood would argue rather for than against the theory of the maintenance of arterial tonicity by the alkaline salts. The difficulty experienced in recovering after a circulation of the bicarbonate and chloride favors the same theory and recalls a phenomenon often witnessed at the beginning of a circulation experiment. When the normal salt solution is being first admitted to the vessels and is washing out the animal's blood, it is not rare to have great irregularities in the pulse-curve, with an exceedingly high average arterial pressure—the actual accompaniments of a recovery from bicarbonate and chloride. As the washing out of the blood proceeds, the pressure falls to a lower level and remains uniform.

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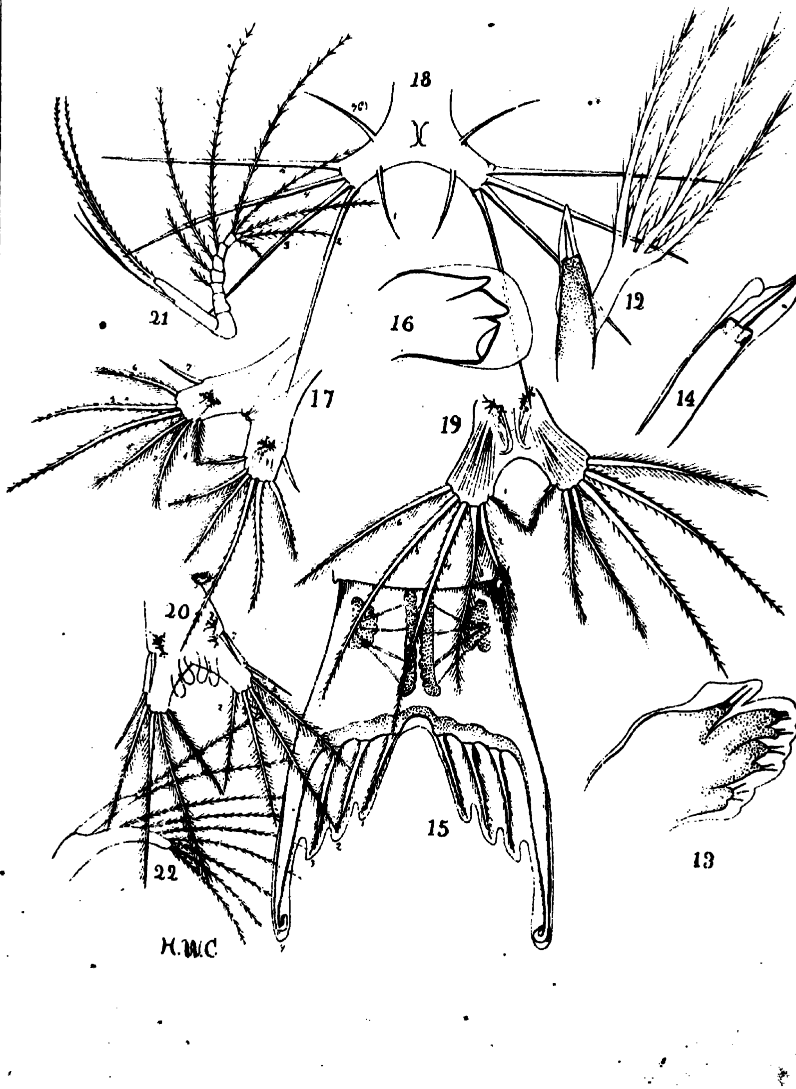

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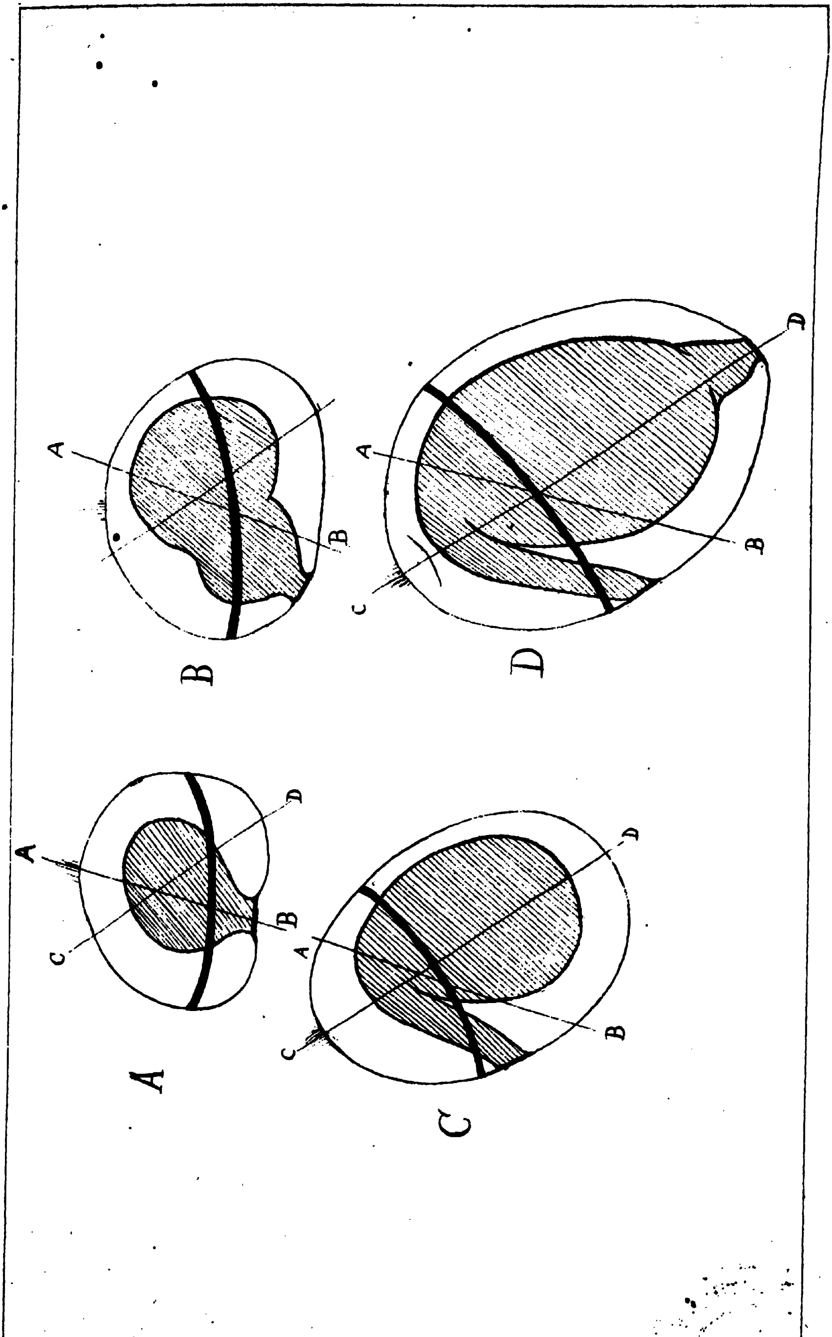

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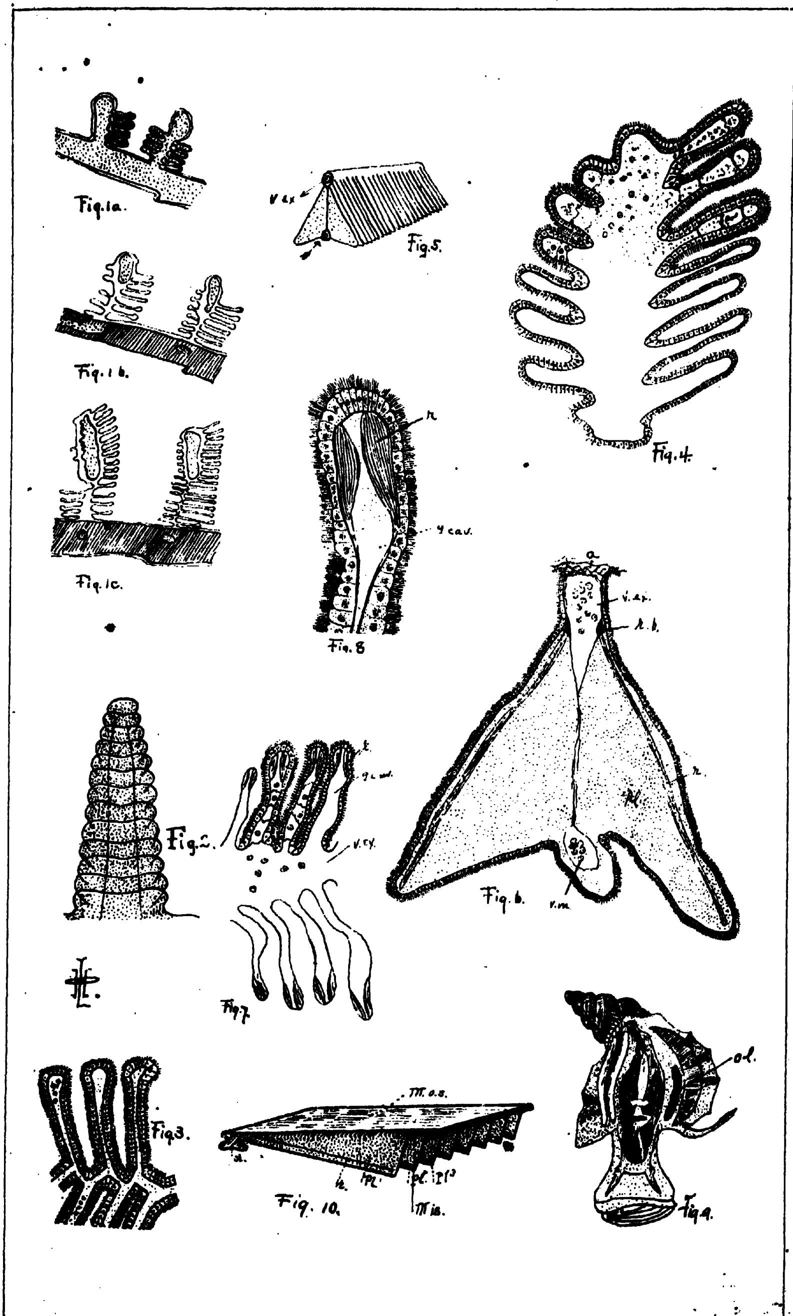

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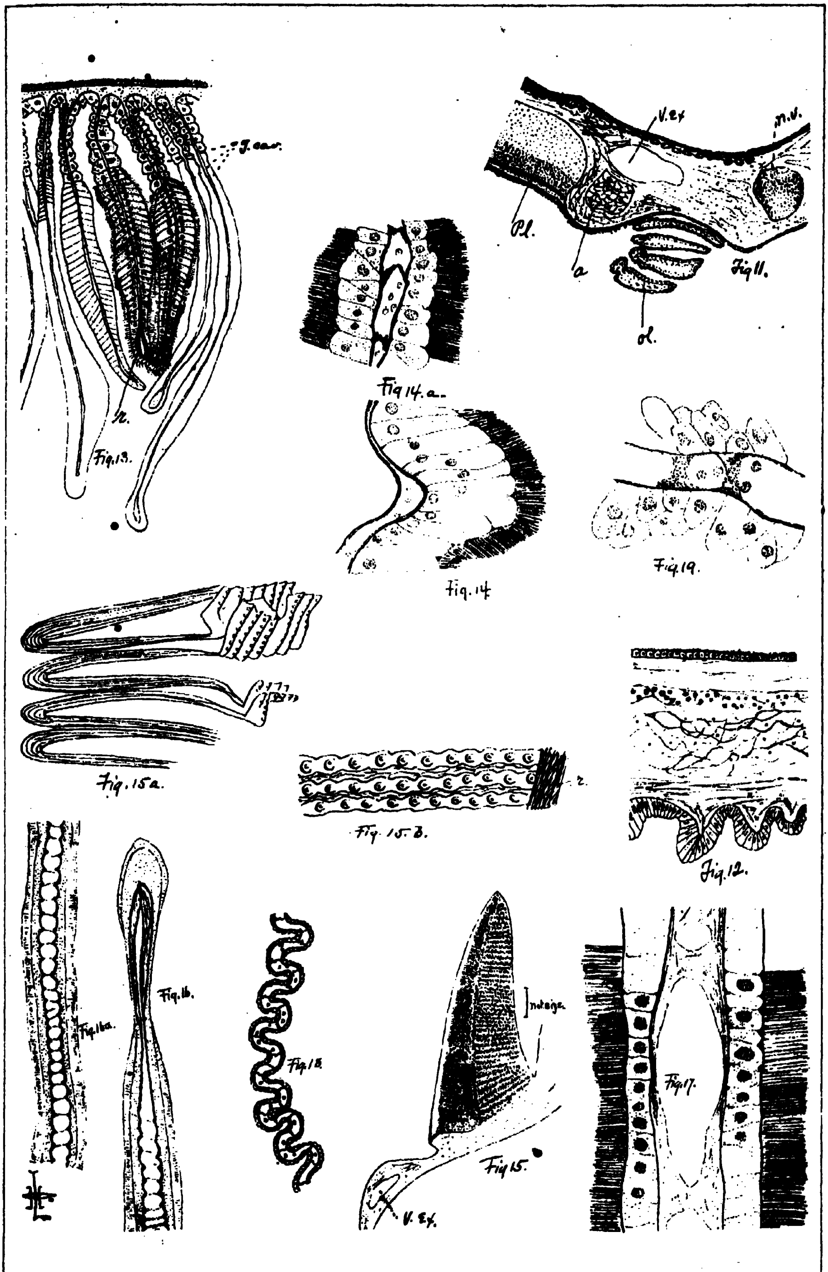

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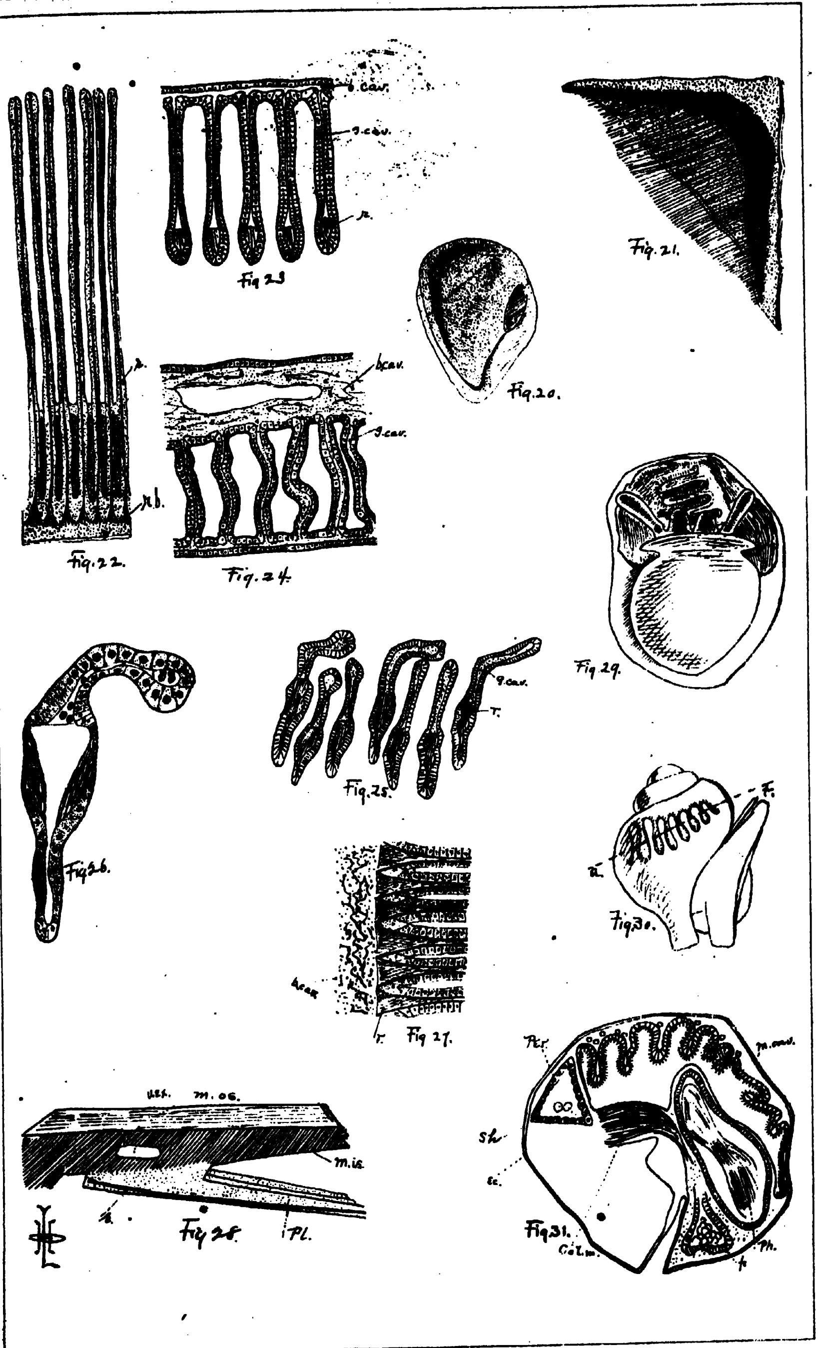

b.cav.
g.cav.
r.
Fig. 23
Fig. 21.
Fig. 20.
r.
r.b.
Fig. 22.
b.cav.
g.cav.
Fig. 24.
Fig. 29.
g.cav.
T.
Fig. 25.
Fig. 26.
F.
a.
Fig. 30.
b.cav.
T.
Fig. 27.
Pcr.
m.cav.
sh.
ec.
Fig. 31.
Col.m.
Ph.
f.
m.os.
m.is.
a.
Fig. 28.
Pl.